University of Southern Denmark

Faculty of Engineering

PhD Thesis:

'Identification and quantification of odorants from livestock buildings'

Submitted by:

'Nawaf Abu-Khalaf'

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‘Identification and quantification of odorants from livestock buildings’

Submitted by: Nawaf Abu-Khalaf
Department of Biochemistry and Molecular Biology (BMB)
Faculty of Engineering
University of Southern Denmark

Supervisor: Associate Professor: Jens Jørgen Lønsmann Iversen
Department of Biochemistry and Molecular Biology (BMB)
Faculty of Engineering
University of Southern Denmark
Campusvej 55
DK-5230, Odense M
Denmark

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Dedication

To my parents & family; and

To everyone, who helped and supported me to reach this important stage in my life.

'We can't change the direction of the wind, but we can adjust our sails'

(Japanese Saying)

'If we knew what it was we were doing, it would not be called research, would it?'

Albert Einstein (1879-1955)

'In order to move forward, you sometimes have to take a step back'

(http://www.sciencedirect.com)

'To invent, you need a good imagination, and a pile of junk'

(Thomas Edison)

'Make everything as simple as possible - but not simpler'

(Einstein)

'The secret of success is to know something nobody else knows'

(Aristotle Onassis, 1906-1975)

Preface

This study is submitted in order to achieve a doctor of philosophy (PhD) degree from the Department of Biochemistry and Molecular Biology (BMB), Faculty of Engineering, in the University of Southern Denmark, Odense, Denmark.

The research work was carried out in the Technical Microbiology group in the BMB department. This PhD program was financed by the University of Southern Denmark (50%), the Danish Research Agency and the Danish Ministry of Food, Agriculture and Fisheries (50%).

This study is a part of a project called 'Absorption in water droplets of odours, ammonia and dust from livestock buildings'. The participants in the project are Danish Institute of Agricultural Sciences / Department of Agricultural Engineering, Aalborg University, Danish Bacon & Meat Council, Solum Group and Turbovent A/S. This project is one of several joint research projects, under the research programme 'Sustainable Technology in Agriculture', financed by the Danish Research Agency and the Danish Ministry of Food, Agriculture and Fisheries. The projects are coordinated by the 'Danish Agricultural Network in Engineering and Technology (DaNet)'.

The odour emission from livestock buildings is causing many environmental and health problems. Biological methods, which are environmentally friendly, are the preferred techniques for reducing odours emitted from livestock buildings. The bioscrubber is one of the biological methods. It comprises an absorption column (air wet scrubber), in which the polluted air stream is washed by water droplets, and a bioreactor (water purification module), which cleans and recycles the washing water coming from the absorption column. Characterization of a mixture of odorants, in absorption column or in bioreactor, gives information about the odorants dissolved in water and the performance of the bioreactor.

The aim of this study was identification and quantification of representative mixtures of key odorants in bioscrubbers of livestock buildings. During this research, the identification and quantification of odorants were carried out by two analytical equipments: (1) gas chromatography (GC), and (2) electronic tongue (ET). GC was used off-line. The ET is a relatively new technology. It is an analytical instrument containing an array of chemical sensors, with partial specificity for different components in liquid media and an appropriate pattern recognition and/or multivariate calibration tool for identification and quantification of simple and complex solutions. It analyses the compounds in liquid media with high sensitivity.

Both analytical equipments identified and quantified some of the investigated key odorants. Also, ET showed a high potential as an on-line sensor for odorants.

This thesis is based on literature review and experimental work. The chapters in the beginning describe the general background of the study, followed by three articles. These articles were submitted to several international journals, and they will give a comprehensive idea about the results of this scientific research.

Acknowledgments

I am greatly indebted to many people for their support and encouragement during the period where this work was done.

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Many thanks to Kim F. Haselmann, from the Chemistry Department, for his cooperation and help during the study, to Dimitriy Kirsanov, for his cooperation and help in issues related to the electronic tongue, and to Morgane Lamote for the help and discussion of the thesis.

I would like also to express my thanks to some people in The Royal Veterinary and Agricultural University (KVL) in Copenhagen, who supported and helped me to reach this stage. Without their help and support, I would not be able to be here and do this research. I would like to express my thanks especially to: Brian Dennis, head of Department of Agricultural Sciences; Bent S. Bennedsen, my supervisor during my M.Sc. degree in the AgroTechnology section; Simon Blackmore, former head of AgroTechnology section, and all the other

colleagues and friends who I enjoyed spending time with in AgroTechnology group / Department of Agricultural Sciences / KVL, as a master student and a research assistant.

Thank you all very much.

Odense, Denmark

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Nawaf Abu-Khalaf

Abstract

The odour emitted from livestock buildings is responsible for many environmental and health problems in the society. The biological methods are favourite techniques to reduce odours. These methods are environmentally friendly. The bioscrubber is one of the biological methods. It comprises an absorption column (air wet scrubber), in which the polluted air stream is washed by water droplets, and a bioreactor (water purification module), which cleans and recycles the washing water coming from the absorption column.

Characterization of a mixture of odorants, in absorption column or in bioreactor, is necessary in the optimization of the bioscrubber. In livestock buildings, there are huge numbers of odorants. A representative selection of these odorants, called key odorants, was used in this study. The key odorants were selected to represent a variety of chemical groups.

Two analytical equipments: gas chromatography and electronic tongue (*i.e.* a sensor array) were used for characterization of odorants.

Gas chromatography-flame ionisation detection (GC-FID) was utilized as an off-line method for characterization of key odorants in the air wet scrubber. Two methods were used before injection of key odorants into the GC-FID: direct aqueous injection (DAI) and solid phase extraction (SPE). Both DAI and SPE methods were efficient for identification of odorants in the air wet scrubber. However, DAI is the method of choice for quantification of odorants, because it is simple, fast, requires small volumes only, without pre-concentration and no derivatisation of the compounds is needed before injection into GC. Two odorants, *i.e.* phenol and 1-butanol, were quantified successfully using the DAI method. Their limit of detection and limit of quantification were below literature values for odorants detection limits in livestock buildings.

An electronic tongue (ET) comprising 14 cross-sensitive electrodes was used for identification, quantification and classification of different test mixtures of key odorants. ET is an analytic instrument which includes an array of electrodes with cross-sensitivity, in addition to an appropriate pattern recognition or multivariate calibration tool that is able to recognize qualitatively and quantitatively the composition of both simple and complex solutions. ET has many advantages compared to other methods. The key advantages are rapidity, simplicity, low cost and simultaneous on-line determination of several components of very different chemical properties in the liquid.

The ET was calibrated using 4 different test mixtures. Initially 14 electrodes were investigated in different principal component analysis (PCA), partial least squares (PLS) and back

propagation neural network (BPNN) models. 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. It was seen that eight electrodes were sufficient for all identifications and quantifications of n-butyrate, ammonium and phenolate.

ET has successfully classified different test mixtures of key odorants. The ET was able to distinguish between two test mixtures of key odorants at the same pH with classification rates in the range of 88 - 100%. Classification between the same test mixtures of key odorants at different pH was even higher, 100%. Also, ET classified different test mixtures of key odorants comprising a variety of the chemical groups at pH 6. The average classification rate (ACR) in this case was 81%. The reproducibility of electrodes was better in this case, where the complexity of the mixture was decreased.

Nine electrodes out of 14 were sufficient for all identification, quantification and classification of test mixtures of key odorants. The decreased, but sufficient number of electrodes improved the performance of the ET since the standard deviation and relative standard deviation of measurements in triplicates decreased in comparison with the array comprising 14 electrodes.

Further research with more cross-sensitive electrodes is needed. However, the results indicate that ET has a high potential as an on-line sensor for measurement of odorants in livestock buildings and as a prerequisite for control of emission from livestock buildings. Moreover, ET might be used as an alarm system for which there is a demand.

Abstract 'in Danish'

Luftbåren forurening fra intensiv husdyrproduktion er den vigtigste begrænsende faktor for omfanget af denne produktion. De forurenende forbindelser kan fjernes helt eller delvist ved adsorption i en væskefase i form af en aerosol, dannet i små dyser, monteret i ventilationskanaler i stalde. Efterfølgende renses væsken i et biofilter, før den returneres til ventilationskanalen.

Bestemmelse af representative, forurenende kemiske forbindelser er en forudsætning for optimering af adsorption og biologisk nedbrydning. Blandinger af karakteristiske lugtstoffer blev analyseret uden (direkte vandig injektion) og med (faststof ekstration) prøveforberedelse ved flammejonisationsgaskromatografi. Fenol og 1-butanol blev ved begge metoder bestemt i koncentrationer under litteraturværdier for koncentrationer i staldbygninger. Direkte vandig injektion blev foretrukket, da prøveforberedelse blev undgået.

En elektronisk tunge, bestående af 14 elektroder med krydssensitivitet overfor hovedsageligt ladede forbindelser, blev testet for at identificere, kvantificere og klassificere forskellige blandinger af karakteristiske lugtstoffer. Signalerne fra alle elektroder blev individuelt analyseret ved statitiske metoder, herunder mønstergenkendelse og multivariat dataanalyse. Den elektroniske tunge har mange fordele sammenlignet med andre analysemetoder: Den er hurtig, ukompliceret og først og fremmest en on-line metode, der kan karakterisere selv komplekse blandinger. Den elektroniske tunge blev kalibreret ved 'principal component analysis' (PCA) samt 'partial least squares' (PLS) og 'back propagation neural network' (BPNN). Ved disse analyser kunne antallet af elektroder begrænses fra 14 til 8 ved kvantificering af ammonium, n-butyrat og fenolat uden tab af analysesikkerhed. Forskellige blandinger af de karakteristiske lugtstoffer blev med 8 elektroder kvantificeret med følsomheder svarende til gaskromatografens og med klassifikationsrater fra 88 til 100%. Standardafvigelser og relative standardafvigelser var lavere for det begrænsede antal elektroder sammenlignet med den elektroniske tunge bestående af 14 elektroder.

Resultaterne viser, at den elektroniske tunge har et stort potentiale som en on-line metode til at karakterisere sammensætningen af staldluft og –lugt. Herved kunne vurderingen af lugtgener blive mere objektive. Endelig er en kvantificering af karakteristiske lugtstoffer en forudsætning for optimering af installationer til begrænsning af lugtgener samt udledning af ammoniak fra staldbygninger, hvilket er en af nutidens største miljøteknologiske udfordringer.

Contents

List of abbreviations

ACR	Average classification rate
ANN	Artificial neural network
BPNN	Back propagation neural network
BTEX	Benzene, toluene, ethylbenzene and xylenes
CH_4	Methane gas
CO_2	Carbon dioxide
CV	Coefficient of variation
D/T	Dilution to threshold
DAI	Direct aqueous injection
DaNet	Danish agricultural network in engineering and technology
EN	Electronic nose
ET	Electronic tongue
EU	European union
FID	Flame ionization detector
FIDO	Frequency, intensity, duration and offensiveness
FIM	Fixed interference method
GC	Gas chromatography
GC-MS	Gas chromatography-mass spectrometry
H	Henry's constant
H_2S	Hydrogen sulphide
HPLC	High performance liquid chromatography
ISE	Ion-selective electrode
K_{AW}	Dimensionless air-water partition coefficient
LOD	Limit of detection
log p	Octanol-water partition coefficient
LOQ	Limit of quantification
MLP	Multi-layer perceptrons
MLR	Multiple linear regression
mV	Millivolt
MVDA	Multivariate data analysis
NH_3	Ammonia
NIPALS	Non-linear iterative partial least squares
OU	Odour unit
P&T	Purge and trap

PC	Principal component
PCA	Principal component analysis
PCR	Principal component regression
PLS	Partial least squares
PLS-DA	Partial least squares-discrimination analysis
r	Correlation coefficient
RMSEC	Root mean square error of calibration
RMSEP	Root mean square error of prediction
RPD	Ratio of standard error of performance to standard deviation
RSD	Relative standard deviation
SDev	Standard deviation
SIMCA	Soft independent modelling of class analogy
SOM	Self organizing map (*i.e.* Kohonen net)
SPE	Solid phase extraction
SPME	Solid phase micro extraction
SSM	Separate solution method
SVD	Singular value decomposition
TON	Threshold odour number
VFA	Volatile fatty acid

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List of papers

1. Nawaf Abu-Khalaf, Kim F. Haselmann, Jens Jørgen Lønsmann Iversen

Identification and quantification of odorants in an air wet scrubber using direct aqueous injection-gas chromatography (DAI-GC) and solid phase extraction-gas chromatography (SPE-GC)

Submitted to: Journal of Chromatography A

2. Nawaf Abu-Khalaf, Jens Jørgen Lønsmann Iversen

Calibration of a sensor array (an electronic tongue) for identification and quantification of odorants from livestock buildings

Submitted to: Analytical Letters

3. Nawaf Abu-Khalaf, Jens Jørgen Lønsmann Iversen

Classification of mixtures of odorants from livestock buildings by a sensor array (an electronic tongue)

Submitted to: Analytical Letters

4. Another article is in preparation now. Unfortunately, it will not be included in this thesis. This is because the authors, who are the partners in the project, are waiting for the official end of the project, so they can publish the main ideas and results

Glenn Tubbert, Morten Øgendahl, Nawaf Abu-Khalaf, Ivar Lund, Thomas K. Hansen, Preben Dahl, Jens Jørgen Lønsmann Iversen, Thomas J. Condra, Hisamitsu Takai

Design and development of wet scrubber for odour control in livestock buildings

In preparation.

1. Introduction

Livestock is one of Denmark's largest industries. Denmark has become a major contributor to technological advances within this field, and it is very famous worldwide for its agricultural technology, *e.g.* milk, cheese and meat production. In Denmark, there were 16073 cow farms, 9994 pigs farms, 3288 poultry farms and 2668 sheep farms in 2004 (StatBank, 2006).

The dairy sector is important in Denmark. The total number of cattle in Denmark is about 1.9 million. Of these, about 0.6 million are dairy cows. About 4.6 million tonnes of milk are produced annually. On average, each dairy farmer keeps approximately 62 cows. Each yielding 7600 kg milk yearly. Production of broilers amounts to approximately 134 million Danish kroner (about 20 million American dollars) yearly. A small production of turkeys, ducks and other poultry are also present. About 210 thousands tonnes of poultry meat is produced yearly. Two-thirds is exported to foreign markets. There are 3.7 million egg-laying hens, producing 74 million eggs annually. This amount covers the national consumption of eggs (Danish Agricultural Council, 2006).

Denmark is one of the world's leading exporters of pork. It is the fifth largest pig meat producer in the European Union (EU); after Germany, Spain, France and Netherlands; but it is the largest exporter. In 1999 the exports of pig meat products accounted for 6.2% of the total value of Danish exports and 46.3% of total agricultural exports. The pig meat accounts for 47% of the world meat production compared to 24% for poultry and 29% to beef. Pig meat accounts for 49% of total meat consumption within the EU (Lara *et al.,* 2002).

The odour problem is an important environmental pollution issue (Yuwono and Lammers, 2004). Livestock buildings are one of the main sources of the odours from the animal production (Carney and Dodd, 1989). It is foreseen that in the coming years large farms will have to significantly reduce the output of odours, ammonia and dust emission to the surrounding area from their livestocks. This is because of the legislation restriction, near-neighbour pressure and environmental issues (Gostelow *et al.,* 2001; Schiffman, 1998). On the other hand, this reduction of odour, ammonia and dust emission will increase the scale and profit of animal productions. This is because the regulation in Denmark enforces a minimum distance of 300 meter as a buffer zone between livestock buildings and neighbouring houses. This buffer zone affects one fourth (25%) of livestock producers in Denmark (DaNET, 2004). There are several methods for reducing odours. These include physical, chemical and biological methods. The biological methods are inexpensive, simple to operate and environmentally friendly (Revah and Morgan-Sagastume, 2005). One of the biological methods is the bioscrubber.

The bioscrubber consists of two main parts: an absorption column (air wet scrubber) and a bioreactor (water purification module). In the livestock building, the absorption column and the bioreactor are place inside the ventilation chimney and at floor level, respectively. In the absorption column, odour substances (odorants), ammonia and dust particles are absorbed by water droplets. Water droplets are provided to the absorption column through water nozzles, who receive water recycled from the bioreactor. The bioreactor can supply cleaned water for several absorption columns. The bioreactor contains microorganisms that metabolize different substances. This results in the production of biomass, water and CO_2 (Revah and Morgan-Sagastume, 2005).

Bioscrubber looks like a good method for Danish livestock buildings, since more than 90% of the Danish poultry and pig houses are estimated to use mechanical ventilation systems, in which the exhaust air is discharged through ventilation chimney. An air cleaning system mounted in a ventilation chimney would therefore be an effective technical solution for the reduction of odour emissions from this type of livestock building (DaNET, 2004).

Identification and/or quantification of a mixture of odorants in absorption column or in bioreactor, gives information about the absorbed odorants and the efficiency of the bioreactor. Also, it allows designers or operators to make the right decisions related to the choice of technique, modifications, etc.

Identification and/or quantification are carried out by different analytical equipments, which work off-line or on-line. Off-line methods include gas chromatography (GC), gas chromatography-mass spectrometry (GC-MS), etc. The on-line method might be achieved by using an electronic tongue (ET); this is an analytical instrument containing an array of chemical sensors, with partial specificity for different components in liquid media and an appropriate pattern recognition and/or multivariate calibration tool for identification and quantification of simple and complex solutions (Vlasov *et al.,* 2005). The ET is a new technology, and it was known for about 10-15 years. There are many advantages in using ET compared to other analytical methods. The key advantages are rapidity, simplicity, low cost and simultaneous on-line determination of several components in the liquid (Legin *et al.,* 2004a). ET was used in many applications.

This study will investigate the possibility of identification and quantification of odorants in bioscrubbers of livestock buildings, using two analytical methods: gas chromatography and ET. However, due to the presence of huge amount of odorants in livestock buildings (O'Neil and Philips, 1992; Schiffman *et al.,* 2001), a representative selection of these odorants was

used in this study, and we called them key odorants. They were chosen from different chemical groups and due to their contribution in the odour nuisance problem.

This is the first study related to use ET for identification and/or quantification of odorants in bioscrubbers of livestock buildings. It is a step forward in a long process aiming to build an on-line sensor of odorants. This study contributes to both sensor and environmental technology.

The following chapters will give a background related to odour, electronic tongue and multivariate data analysis. The latter is used to convolute the complicated signals produced by ET.

2. Aim and objectives

The aim of this research was to investigate the possibility of using ET (*i.e*. a sensor array) as an on-line sensor for identification and/or quantification of odorants in bioscrubbers of livestock buildings. To achieve this aim, the following objectives were formulated:

- To identify and/or quantify key odorants using an off-line method. GC was used. The off-line method might be used as a reference method for an on-line technique, *i.e.* ET,
- To investigate the possibility of using ET to identify and/or quantify key odorants,
- To investigate the possibility of using ET to classify different mixtures of key odorants, and
- To simplify ET, by decreasing the number of electrodes from the maximum number to fewer electrodes that are able to model key odorants and classify different test mixtures of key odorants, without any loss of analytical information.

3. Odours

To be able to reduce or control odours, it is important to study and understand them. This chapter handles a mini literature review related to odours. It covers the general aspects of odours and emphasize mainly on agricultural odours.

3.1. General concepts

Smell is the most sophisticated sensory system for mammals, including human beings (Dalton, 2003). Generally, odour or malodour refers to unpleasant smell (Yuwono and Lammers, 2004). There is no universal definition of odour (Mackie *et al.,* 1998). However, odour can be defined as the perceived effect of one or more odorants as detected and interpreted by the olfactory system (Gostelow *et al.,* 2001; Mackie *et al.,* 1998; Schiffman *et al.,* 2001). Odorants are the compounds responsible for imparting an odour, and their molecules mass is generally between 30 to 300 Daltons (Persaud *et al.,* 1996b; Sarig, 2000). The molecular structures of odorants are very diverse (Persaud *et al.,* 1996b). The relation between odorant properties and odour perception is not clear. This is due to the lack of a full theory of olfaction (Gostelow *et al.,* 2001).

Detection of odours is a very complicated process (Dalton, 2003). The human olfactory system consists of three parts: olfactory epithelium, the olfactory bulb and the olfactory cortex. The olfactory epithelium is an area of approximately 5 cm^2 located in the upper nasal cavity, below the eyes. It contains about 10^7 to 10^8 receptor cells. The receptor cells connect via olfactory neurones to the olfactory bulb at the base of the brain. There, the pre-processing of the electrical output from the receptor cells takes place before passing to the olfactory cortex, where further processing takes place in the higher order olfactory structure of the central nervous system. The receptors have a broad response with a large overlap between different classes. Humans are just able to name few odours by analogy, *e.g.* it smells sweaty, fishy, etc. (Gostelow *et al.,* 2001; Mackie *et al.,* 1998).

The 2004 Nobel Prize winner in medicine, Richard Axel and Linda B. Buck, have contributed greatly to the understanding of the mechanisms involved in olfaction. They cloned olfactory receptors, and showed that they belong to the family of G protein coupled receptors. By analyzing rat DNA, they estimated that there were approximately 1000 different genes for olfactory receptors in the mammalian genome. That research opened the door to the genetic and molecular analysis of the mechanisms of olfaction. It was suggested that combined stimulation from different types of receptors can enable the brain to identify a large number of different odours (Nobel Prize, 2004). The humans are capable of recognizing and remembering 10000 odours or more (Mackie *et al.,* 1998; Nobel Prize, 2004). The

deep understanding of Axel and Buck theory is beyond the scope of this study. On the other hand, it just shows that the olfaction system is still a complicated scientific issue until recent days.

Odorant molecules emanating from different sources must be sufficiently volatile to arrive at the olfactory receptors in the nose. They should also be partly soluble in water, so that they can pass through the nasal mucus to the olfactory epithelium, and they should be soluble in lipids, so that they can pass through the lipid layer that forms the surface of the olfactory organ, *i.e.* the nose (Persaud *et al.,* 1996b; Sarig, 2000). It is estimated that 10% of the air that we breathe through our nose actually reaches the olfactory epithelium (Dalton, 2003). The perception of people toward odour is highly individually. It depends on many factors, such as gender, age, general health, smoking habits and repeated exposure to an odorant (Gostelow *et al.,* 2001).

3.2. Agricultural odours

The sources of odours from livestock units are: (1) manure, (2) ventilation exhaust air from farm building, (3) animals, and (4) feed (Carney and Dodd, 1989). Nevertheless, the odour emissions from animals and feed are considered low when compared to manure and ventilation exhaust air odours. In Europe, it was estimated that the total odour emissions from animal facilities are made up of 50% from indoor exhaust air, 25% from manure storage and 25% from manure transport and spreading (Klooster Van't and Voermans (1993) cited in Sheridan *et al.* (2002)). Odorants causing malodour are produced by micro-organisms decomposing various substances like undigested feed particle in manure (Mackie *et al.,* 1998). The emission rates of odours from livestock operations are dependent on many factors. Some of these factors are temperature, humidity, time of the year and day, weather condition, ventilation rates, wind force, housing type, manure properties and animal species (Jacobson *et al.,* 1999). Dust is an important odour and ammonia carrier. Dust concentration and emissions is dependent on housing type and animal species. The highest dust concentrations are found in poultry housing followed by pig and cattle (Takai *et al.,* 1998; Takai *et al.,* 2002). Some of the methods to reduce dust are spraying oil or oil-water mixture inside the building, as well as mechanical or electrical dust removal from air head space (Zhang *et al.,* 2002).

3.3. Odours and health

Odours may lead to health problems, including physiological and psychological conditions. They can potentially affect memory (Schiffman, 1998). It was also found that the

neighbours of livestock buildings suffer more from depression, negative emotions, greater mood disturbance, more tension, an overall feeling of less vigour, anger, fatigue, and confusion than people living far away from livestock buildings (Schiffman *et al.,* 1995). Moreover, odours cause a decreased quality of life for the vicinity people, *e.g.* they could not enjoy the nice weather in their home gardens (Wing and Wolf, 2000). A significant percentage of agricultural workers have respiratory diseases from the long term exposure to dust and odours (Kirkhorn and Garry, 2000). These symptoms were mainly found in and near pig farms. The odours from the livestock buildings are not toxic. However, livestock buildings should be equipped with proper ventilation systems, and the persons (farmers) who are working inside them should have fresh air outside the buildings during working hours (Dalton, 2003; Gostelow *et al.,* 2001). High level of odour in pig production facilities affects the animals' health. The growth decrease and the susceptibility to diseases increase (Mackie *et al.,* 1998).

3.4. Odour measurements

Odour nuisance (*i.e.* irritation, annoyance) is one of the most important factors in odour environmental problems. Odour nuisance is generally defined by the 'FIDO' factors. FIDO stands for: Frequency, Intensity, Duration and Offensiveness. Frequency refers to the number of times an odour occurs. Intensity refers to the strength of an odour. Duration refers to the period of time an odour is encountered, and offensiveness refers to the character or hedonic tone of the odour (Mackie *et al.,* 1998; O'Neil and Philips, 1992). Offensiveness measurement is more subjective (individual) than intensity measurement. People perception of the offensiveness of an odour differ more than their perception of odour intensity, and it is difficult to differentiate between degrees of offensiveness (Misselbrook *et al.,* 1993).

Odour intensity is the key factor in quantification of odour nuisance problems. Generally, the odour intensity from animal housing air decreases from pig through poultry to cattle (Hartung, 1992; Jongerbreur *et al.,* 2003). Hartung (1992) found that the intensity is affected by the age of the animals, the type of housing and the reason for which they are being kept.

Direct and indirect methods have been developed for measuring odour intensity. Direct (sensory or olfactometric) methods involve the use of about 4-16 trained persons in a panel, using their nose as a detector. Indirect (analytical) methods measure the concentration of volatile odorants in air, and if possible correlate the measurements to direct observations (Le *et al.,* 2005; Mackie *et al.,* 1998; Rappert and Muller, 2005). So, the analytical measure-

ments refer to odorants, while the sensory measurements refer to odours (Gostelow *et al.,* 2001; Mackie *et al.,* 1998).

3.4.1. Olfactometric methods

The olfactometric method uses two major techniques: scaling and dilution.

The principle of scaling measurements is to vary the odour concentration and thus vary perceived intensity. Scaling involves: (a) rating the odour intensity on an arbitrary scale (using a seven points rating scale that ranges from no odour to extremely strong), or (b) referencing odour intensity to the intensity of a known substance (Le *et al.,* 2005; Mackie *et al.,* 1998; Rappert and Muller, 2005). The most commonly used reference compound is n-butanol (C_4H_9OH). This is mainly due to its stability, availability in high purity, relatively non-toxic and has a reasonably agreeable odour that is unrelated to most other odours of concern (Mackie *et al.,* 1998; Schulte, 2000). The scaling methods are simple, easy and do not need elaborated equipment (Mackie *et al.,* 1998).

The dilution methods involve presenting the panellists with a range of dilutions of the odours samples in liquid or vapour form to determine odour threshold or detectability. The results from the panellists are expressed as a dimensionless ratio, like threshold odour numbers (TON) or dilution to threshold (D/T). So, odour concentrations derived by the threshold olfactometry are dimensionlessly expressed (Gostelow *et al.,* 2001). However, since the odour concentration from olfactometry is the mostly commonly used parameter for signifying the intensity (strength) of odours (Le *et al.,* 2005), the dimensionless ratio has been expressed as a physical concentration, and it is called odour units (OU) or odour units per cubic meter (OU/m^3) (Gostelow *et al.,* 2001). The definition of one odour unit is the amount of the odour, which when diluted in 1 m^3 of air, can just be distinguished from clean air by half (50%) of the members of an odour panel (Le *et al.,* 2005). Here is an example about OU: if an odour sample, collected from the farm, needs to be diluted 70 times before 50% of the persons on the odour panel can detect the odour and 50% can't, then the odour stream has an odour intensity of 70 OU or OU/m^3. Liquid dilution has mainly been used in the estimation of odour in water and wastewater treatment effluents (Mackie *et al.,* 1998).

The intensity and odorant concentration are correlated. The perceived intensity increases with increasing odorant concentration. Nevertheless, the correlation is not linear (Gostelow *et al.,* 2001). There are two laws explaining this relationship: Weber-Fechner law and Steven's law. The Weber-Fechner law produces a linear plot of intensity versus logarithm of odorant concentration. Steven's law produces a linear plot of intensity logarithm versus

logarithm of odorant concentration (Gostelow *et al.,* 2001; Le *et al.,* 2005). However, both laws give almost the same results with respect to intensity (Misselbrook *et al.,* 1993).

Dilution methods can be static or dynamic. Static dilution involved the mixing of known volumes (m^3) of odours and odour free air. Dynamic dilution involves the mixing of known flow rates (m^3/sec). Dynamic dilution has some advantages over static dilution, as (1) minimizing the effects of sample adsorption to the internal surfaces of the instrument, and (2) delivering the sample to the sniffing port at a constant flow, which helps to improve the repeatability of the results (Gostelow *et al.,* 2001). Dilution methods are more objective than scaling methods. The use of a reference (*e.g.* n-butanol) is useful in both scaling and dilution methods, since it will help in comparing values from different panellists (Mackie *et al.,* 1998).

A device called a scentometer is used for direct field measurements, to determine the thresholds dilution. It is a small device that can be carried by hand. The principle is similar to the dilution method of olfactometric. The procedure is to vary proportions of ambient air, *i.e.* odours, which are drawn through an activated carbon filter, *i.e.* odour free air, that introduced to two nasal ports for the nose of a trained and experienced person. The ratio of the ambient air to filtered air at which an individual detects an odour is the dilution threshold (Mackie *et al.,* 1998; Rappert and Muller, 2005). The most common methods for measuring odour intensity for research purposes are dilution olfactometer and scentometer (Mackie *et al.,* 1998; Rappert and Muller, 2005).

For practical applications, values of OU higher than 7 will create odour complaints (Schulte, 2000). A serious nuisance is expected at a value of 31 OU (Gostelow *et al.,* 2001). However, there are some limitations of the sensory (olfactometry) methods for measuring odour intensity, as expensive, labour intensive and time consuming. Moreover, these methods are subjective and personality variation in sensitivity to different odours, quick saturation of olfactory senses, fatigue as a result of adaptation and climatic variables changes when measuring odours under field conditions (Le *et al.,* 2005; Mackie *et al.,* 1998; Rappert and Muller, 2005). Gostelow and Parsons (2000) stated that even with a lot of standardization procedures presented these days, the olfactometry is not a precise measurement. Furthermore, until recent days, no individual compound is used as an indicator to predict the olfactory responds (Zhang *et al.,* 2002). Consequently, there is a need for other methods for measuring odours. This might be done by indirect analytical methods, in which separation, identification and quantification of odorants can be carried out.

3.4.2. Analytical methods

Analytical measurements refer to odorants, while sensory measurements refer to odours (Gostelow *et al.,* 2001; Mackie *et al.,* 1998). Despite that the intensity of odour, as it will be perceived by humans, is not related to the analytical methods (Mackie *et al.,* 1998; Misselbrook *et al.,* 1993), the advantages of analytical measurements compared to sensory measurements are: (1) objective, (2) repeatable, (3) accurate, (4) automated samples and measurement, and (5) related to the theoretical models of odorant formation or emission (Gostelow *et al.,* 2001; Gostelow and Parsons, 2000; Mackie *et al.,* 1998). The odorants concentration is the most common measurement by analytical methods (Gostelow *et al.,* 2001; Gostelow and Parsons, 2000). Misselbrook (1993) suggested that odours of equal concentration will not necessarily have equal perceived intensity or offensiveness.

Gas chromatography (GC) is the most commonly used analytical technique in separation and identification of volatile compounds (Mackie *et al.,* 1998; Rappert and Muller, 2005). GC is a precise method and might be used off-line, *in situ* and for continuous measurements (Rappert and Muller, 2005). The peak area and the height are used to quantify the concentration of each compound. The compounds are separated by injecting a sample into specific columns. They are separated in the column according to their vapour pressure and solubility (Mackie *et al.,* 1998). Figure 1 shows a schematic diagram of a GC.

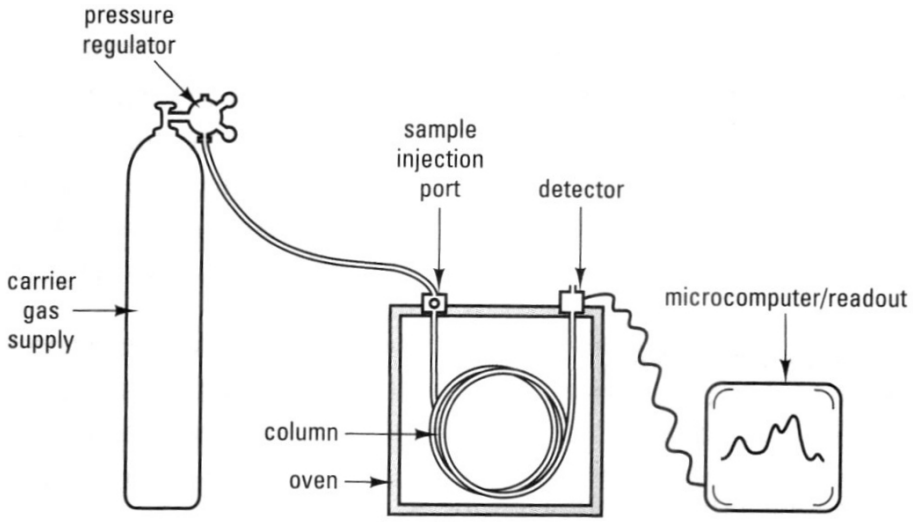

Figure 1. Schematic diagram of gas chromatography (Source: Reed at al., 1998)

There are specific detectors for GC that are sensitive to certain types of compounds. Flame ionization detector (FID) is a universal detector for a broad range of organic compounds. It consists of a hydrogen/air flame and a collector plate. The effluent from GC column passes through the flame. The flame breaks down organic molecules and generates ions. This creates a small current. The response is proportional to the number of carbon in the analysed

compound. The ions are collected on a biased electrode and produce an electrical signal. The FID is extremely sensitive with a large dynamic range. One of its disadvantages is that it destroys the sample (Reed *et al.,* 1998). Combining GC with mass spectrometry (GC-MS) improves the certainty of the identified compounds. However, it is an expensive device, used just for research rather than for monitoring, does not correlate well with the dilution levels required for detection of odour by a human panel using olfactometry, and the limit of analytical detection may be higher than the thresholds of smell (Bourgeois *et al.,* 2003; Mackie *et al.,* 1998; Rappert and Muller, 2005). Sometimes, GC or GC-MS is coupled with an olfactometry port to link and compare the results between sensory and analytical methods (Wright *et al.,* 2005). GC with different detectors has been used in many applications related to sewage odours (Gostelow *et al.,* 2001). Also, purge and trap (P&T), solid phase micro extraction (SPME) and solvent extraction were used as a separation techniques before GC and/or GC-MS analysis (Kim *et al.,* 2002; Razote *et al.,* 2004; Shin and Ahn, 2004).

The identification and quantification of all the odorants present in a sample is a very difficult process. This is due to two main factors (Gostelow and Parsons, 2000):

1. A large number of odorants are present in the sample (*i.e.* mixture of odorants), and
2. The presence of odorants in very low concentrations.

Moreover, the main obstacle in linking analytical and sensory concentration measurements is the effect of mixtures (Gostelow and Parsons, 2000). Complex odours, which have several individual odorants, smell differently than the individual odorant. It is so difficult to predict the odorants in an odour mixture. The unpleasantness of the odorant mixture increases with the number of odorants in the mixture and the growing concentration of the odorants. However, it is not a linear relationship. The detection threshold for a mixture of odorants is generally lower than for any individual odorants, indicating that a synergistic additivity among odorants is present. Nevertheless, the additivity is hard to predict (Gostelow *et al.,* 2001; Mackie *et al.,* 1998). The perceived intensity for a mixture is likely to be higher than for any individual odorant in the mixture (Gostelow *et al.,* 2001). This contributes to the nuisance problem related to the odours emitted from agricultural productions. Even though the individual odorants rarely exceeds the recognition or irritation threshold (Schiffman *et al.,* 2001).

There are four odour threshold concentrations: the detection threshold, the recognition threshold, the irritation (or nuisance) threshold and the toxic threshold. The detection threshold is the lowest concentration that the odorant can be perceived by the human nose or

by half of an olfactometric test panel. The recognition threshold is the lowest concentration of the odour or odorant where it is possible to recognize the source or odour character, and it is 1.5-10 times of the detection threshold. The irritation threshold is often in the range of recognition threshold, and it is 3-10 times the detection threshold. The toxic threshold is the concentration of the odorant that the toxicological effect of the odorants negatively affects the human health. It is about 500 times the odour threshold of an odorant (Dalton, 2003; Gostelow *et al.,* 2001; Mackie *et al.,* 1998; Schiffman *et al.,* 2001).

3.4.3. Odour and electronic nose (EN)

The use of sensor arrays may be one optional method for correlating intensity and concentration of odorants. Electronic nose (EN) is one of the sensor arrays that were used for identification and quantification of samples presented in gas form. EN is defined as an instrument consisting of an array of partial specificity chemical sensors and an appropriate pattern recognition system capable of recognition odour. It has been used in many quality controls, especially in the food processing industry. It has a high potential to be used in medical applications (Stefan *et al.,* 1999). Also, it has been used in environmental and odour investigation purposes (Hobbs *et al.,* 1995; Persaud *et al.,* 1996a; Stuetz and Nicolas, 2001). EN is commercially available (Nagle *et al.,* 1998). However, Le *et al.* (2005) suggested that EN is still far from implementation in measuring livestock odour. Some problems should be solved before EN can be used in a farm, as large size, periodic calibrations, sensor drift, humidity and temperature dependence, high power consumption and high cost (Bourgeois *et al.,* 2003; Nagle *et al.,* 1998; Stefan *et al.,* 1999).

3.4.4. Key odorants

There are few reviews and studies related to quantification of odorants from livestock buildings. Those studies primarily addressed swine operations (O'Neil and Philips, 1992; Schiffman *et al.,* 2001). There are a huge numbers of odorants in livestock buildings. About 300 different odorants have been identified (Schiffman *et al.,* 2001), many of them have a very small detection threshold value of 1 $\mu g/m^3$ or less (O'Neil and Philips, 1992). Key odorants are usually used in analytical measurements, to reduce the required analytical work and time expenditure needed for analysis. The key odorant should be representive for the diversity of the odorants present in the original odour (Schiffman *et al.,* 2001). Many scientists used a representative compounds to evaluate a new technology or application, and to study odours (Razote *et al.,* 2002; Zahn *et al.,* 2001). In some cases, a particular odorant may be dominant and give an indication of the overall odour concentration. A good example

of that is hydrogen sulphide (H_2S), which is the dominant odorant associated with sewage odours (Gostelow *et al.,* 2001; Gostelow and Parsons, 2000).

Choosing of key odorants is a difficult task, since it is so complicated to correlate the nuisance, offensiveness or intensity to specific odorant compounds. However, many scientific attempts were carried out to suggest the most important odorants, which have the most offensive and intense effects in the different animal production facilities. Barth *et al.* (1974) suggested volatile fatty acids (VFAs), ammonia (NH_3) and H_2S. Schaefer (1977) recommended VFAs, phenol, p-cresol, indole and skatole. Spoelstra (1980) suggested p-cresol and VFAs as indicators of odour offensiveness from animal production facilities. Other odorants like VFAs, phenol, p-cresol and skatole were suggested by Williams and Evan (1981). Four major groups of odorants: VFAs, indoles, phenols and sulphides were recommended by Williams (1984) and Hobbs *et al.* (1997). Mackie *et al.* (1998) and Zhu (2000) divided the main odorants to four groups: VFAs, indoles and phenols, ammonia and volatile amines, and sulphur containing compounds. Branching and long chain VFAs (which contains four to nine carbons: C4-C9) may present a good indication of the offensiveness of the animal odours (Zhu *et al.,* 1999). P-cresol was suggested by Wright *et al.* (2005) as the main indicator of odour offensiveness. Le *et al.* (2005) concluded that p-cresol is the most important compound in odour nuisance problem, followed by indole and skatole.

Despite that many livestock buildings have the same housing systems, there is a huge variation in measurements of odour emission in different locations of livestock buildings. The coefficient of variation (CV) for these measurements is in the range of 7-83% (Le *et al.,* 2005). The literature reviews related to odour concentration in livestock buildings, in the last thirty years, reported a widely range of concentrations among different studies. These differences included the odour threshold, the minimum or the maximum odour concentrations in the studies. The variations are possibly due to: (1) the accuracy of the equipments used, (2) different measuring and sampling methods, (3) different sources of samples, and (4) the rapid change in the animal production system from the seventies until nowadays, which may include housing systems, animal breeds and diet. The best way to reduce the variations in the future is to set and follow the standardization measuring methods (Le *et al.,* 2005).

3.4.5. Correlation between different odorants

The correlation between different odorants and combinations of odorant in livestock buildings are weak (Nimmermark, 2004). Nevertheless, there were some scientific trials to study

this point and find correlations. The correlations may help in linking information collected from the livestock production during research or working.

The correlation between NH_3 and odour in livestock production has been investigated. Pain and Misselbrook (1990) found a positive correlation and Heber *et al.* (1998) found no correlation. On the other hand, Jongerbreur *et al.* (2003) concluded that the correlation between NH_3 and odour emission is not reliable in the livestock production. Hobbs *et al.* (1999) studied the effect of aging pig waste (*i.e.* for 112 days) on odour emission rate. They found that the emissions of phenols, VFAs, H_2S and carbon dioxide (CO_2) showed a decrease, while methane (CH_4) and NH_3 showed an increase with increasing storage period.

Le *et al.* (2005) suggested that: (1) the production of indole and skatole is negatively correlated with each other, (2) methane does not cause odour nuisance. But it plays a considerable a role to the greenhouse effect, (3) VFAs concentrations are negatively correlated with methane production, (4) H_2S emission rate is negatively correlated to the methane emission rate, and (5) NH_3 emission rate correlated positively with methane emission rate.

3.5. Odour reduction methods

There are mainly three methods for odour control and reduction (Revah and Morgan-Sagastume, 2005):

1. Physical methods: *e.g.* adsorption, absorption and condensation.
2. Chemical methods: *e.g.* thermal oxidation (incineration) and plasma technology.

The physical and chemical methods transfer the odour from gas phase to another phase (solid or liquid).

3. Biological methods: they use microorganisms (bacteria, fungi) to degrade gas pollutants, which are transferred into the liquid phase. The microorganisms utilize these molecules as a source of nutrients and energy for growth, producing more biomass and carbon dioxide, water, sulphate, nitrate, etc. as by products. So, the microorganisms convert organic and certain inorganic compounds to less toxic and odourless compounds.

The choice of a particular or a combination of these methods depends on: (1) the investment and maintenance cost, (2) treatment objectives, (3) the nature and complexity of the odours compounds (*e.g.* flow rate, volume, concentration, solubility, intensity, temperature, oxygen content), and (4) site characteristics, *e.g.* operation, maintenance capabilities (Burgess *et al.,* 2001; Revah and Morgan-Sagastume, 2005).

The physical and chemical odour reduction methods are mainly used for waste gas streams where the flow and pollutant concentration are high. Despite that they may have a high efficiency, they are expensive. This is due to their consumption of a large amount of chemicals, demand labour control and maintenance of the equipments (Koe and Yang, 2000; Revah and Morgan-Sagastume, 2005).

The biological methods have the following advantages over the chemical and physical methods (Burgess *et al.,* 2001; Revah and Morgan-Sagastume, 2005): (1) effective at low concentrations, (2) inexpensive and economic, (3) environmentally acceptable, (4) have a good efficiency, (5) need very little energy, (6) simple to operate, (7) applicable for a wide range of pollutants, (8) can be used under normal conditions, *i.e.* temperature, pressure and pH, and (9) pollutants are converted to biomass instead of being transferred to another phase, as in chemical or physical methods where disposal will still be a concern.

The most common configurations of the biological methods are biofilters (most extensively used), biotrickling filters and bioscrubbers. These configurations are often known as bioreactors (Yuwono and Lammers, 2004).

3.5.1. Biofilter

A biofilter is a fixed-bed bioreactor. It is based on a filter media bed filled with a porous moist packing material (*e.g.* wood chips, compost). Microorganisms grow on the surface and gaps of the packing material and they form a biofilm. The efficiency of the biofilm is depending on the environmental conditions and the microbial density. The contaminated gas enters to the biofilter from the bottom or the side. It goes upward through the packing material into which the pollutants are absorbed on the packing material for microbial degradation by the biofilm (Revah and Morgan-Sagastume, 2005).

Despite that the biofilter may be active in degradation of odours, it may produce spores. These spores have a potential respiratory health danger when they are blown and inhaled by the people around (Ogendahl, 2005).

3.5.2. Biotrickling filter

The gas flows through a fixed bed (*e.g.* structured plastics, ceramics), that is irrigated with an aqueous solution continuously. The solution contains the nutrients required by the biological system. The fixed bed has microorganisms, and they form a biofilm. The gas is firstly absorbed by the aqueous film that surrounded the biofilm (biological layers). Then the biodegradation takes place within the biofilm that slowly develops on the fixed bed particles (Delhomenie and Heitz, 2005). The main disadvantage of biotrickling is the accu-

mulation of excess biomass in the filter bed, when the nutrients and water are not properly controlled and also due to time factor. This will lead to decrease in performance and clogging problems (Delhomenie and Heitz, 2005; Revah and Morgan-Sagastume, 2005).

Iranpour *et al.* (2005) reviewed the air pollution control using biofilters and biotrickling filters. They focused mainly on different applications in United States of America. They concluded that both methods are capable of removing odour and H_2S from waste gas. Also, they found that the removal efficiency in the laboratory scale is higher than in the field applications. This is logical and expected, due to complexity of odours in the real life, and studying just only one or two odorants in the laboratory scale.

3.5.3. Bioscrubber

The bioscrubber consists of two units. The first unit is the absorption unit (air wet scrubber), and the second unit is the bioreactor (water purification module). In the absorption unit, gaseous contaminants, that are soluble in water, will transfer to the liquid phase. The absorption unit preferably contain a packed bed. The water that contains the compounds will be cleaned in the bioreactor. The microorganisms present in the bioreactor. The 'degrading' microorganisms and the nutrients needed for their growth and survival are suspended in the aqueous phase in the bioreactor. The effluent leaving the bioreactor is recirculated to the top of the absorber unit. This recirculation will help in absorbing more gaseous contaminants (Delhomenie and Heitz, 2005; Singh *et al.,* 2005). Bioscrubbers were successfully used in many industrial and agricultural applications, and their use is growing (Kraakman, 2005).

The main advantages of the bioscrubbers over the other biological configurations are: (1) easily control of biological parameters (pH, nutrients, temperature, and removal of metabolic products are controlled in the bioreactor), (2) operational stability, (3) low pressure drop, (4) small equipment volume, (5) no clogging problem of packing material, (6) suitable for elimination of water-soluble compounds where pH is an important factor in the elimination process, (7) capable of handling large gas flow rates (about 3000-4000 m/hr), (8) capable of handling pollutant with a concentration less than 5 g/m^3, (9) capability of removing pollutant degradation by washing the bioreactor, (10) low operational cost, and (11) having two separate parts, making it possible to dimension each part separately to adapt to any modification. However, the main disadvantage of bioscrubbers is that they are used for treatment of compounds that only have a high solubility in water, *i.e.* compounds with low dimensionless air-water partition coefficient (K_{AW}). The K_{AW} is the concentration of the compound in the air phase divided by the concentration of the same compound in liquid phase, assuming that the gas and liquid are at equilibrium. Compounds treated by bioscrub-

bers are preferably have K_{AW} less than 0.01. This disadvantage makes bioscrubbers less popular than biofilters. To solve the problem of compounds that are not high soluble in water, there were successful trials to add emulsifying agents to improve their solubility (Burgess *et al.,* 2001; Datta and Allen, 2005; Delhomenie and Heitz, 2005; Singh *et al.,* 2005).

Figure 2 shows a schematic design of a bioscrubber. However, some research just used the absorption column and they called it air scrubbing or air wet scrubber (Melse and Ogink, 2005).

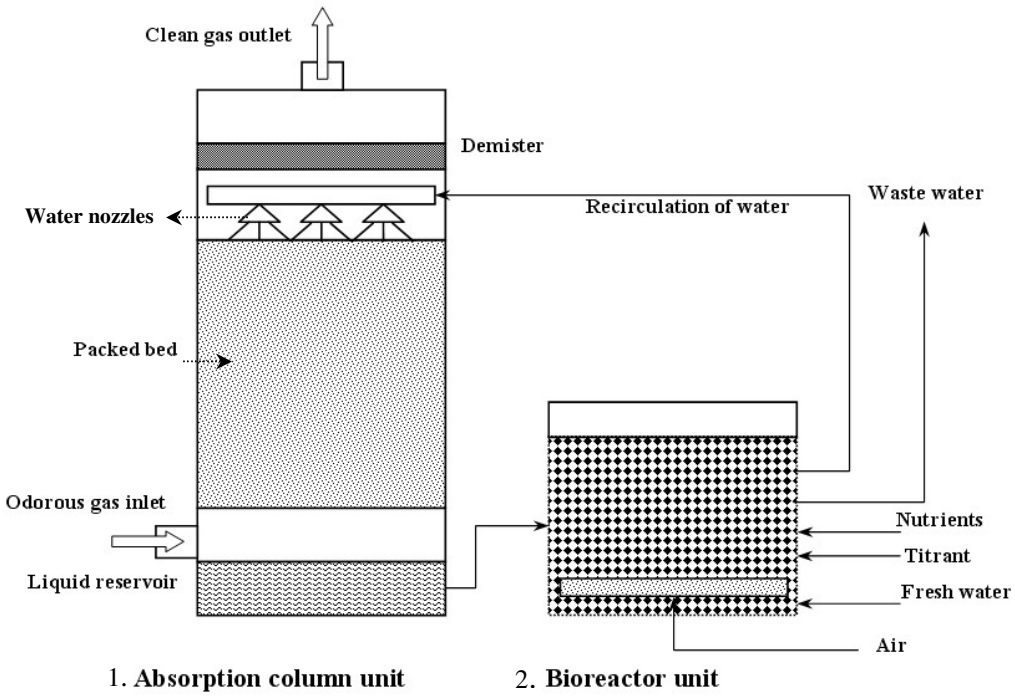

Figure 2. Schematic design of bioscrubber. It contains two units: (1) absorption column, where the odours gas is transferred into liquid phase, and (2) bioreactor, where biodegradation by active microbial culture occurs (adapted from: Singh et al., 2005)

The three types of bioreactors mentioned above have four critical common parameters: (1) pH, which is generally optimum at about seven, (2) temperature, which is between 20 to 40^{o}C, (3) availability of essential, non carbon nutrients (nitrogen, phosphate, potassium, sulphur and micronutrients), and (4) moisture content in the growth media. The main differences are their design and mode of operations (Delhomenie and Heitz, 2005). Table 1 presents the main differences between the three types of bioreactors.

Table 1. Main differences between different biological configurations (Source: Delhomenie and Heitz, 2005; Yuwono and Lammers, 2004)

Characteristics	Biofilter	Biotrickling filter	Bioscrubber
❑ Reactor design	Single reactor	Single reactor	Two reactors
❑ Microorganisms	Fixed	Fixed	Suspended
❑ Liquid phase	- Stationary - The bed is occasionally irrigated with nutrients solution	- Flowing - Continuous trickling over the filter bed - Possible recycling	- Flowing - Continuously distributed - Recycled
❑ Clogging of packing	May occur	May occur	No clogging problem
❑ Removal steps of odours	- In the filter bed - In the biofilm	- In the filter bed - In the biofilm	- Air and gas are separated in the absorption column - Odorants are metabolized by the microorganisms in the bioreactor

Generally, in all bioreactor configurations (*i.e.* biofilter, biotrickling filter and bioscrubber), the reported average removal efficiency of ammonia is higher and has less variations than the reported average removal efficiency of any other odorants. The removal efficiency is the fraction of the odorant removed by the bioreactor expressed in percentage. The high reported variations indicate that the removal of odorants is a difficult problem and no agreement between scientists about different odorants removal efficiency. Still a lot of research should be carried out within odour reduction technologies (Datta and Allen, 2005; Melse and Ogink, 2005).

The pH is an important factor in different bioreactor configurations. This is because the pH has an effect on 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. Most bioreactors have mixed microbial cultures, and the optimum pH levels are in the range of 4-8 (Singh and Ward, 2005). However, most microbial growth occurs near neutral pH (McNevin and Barford, 2000). Cautions should be taken for the fluctuations in pH levels, since they are

generally harmful for microbial activity. Microbial cultures for the different waste gas contaminants treatment were reviewed by Sing and Ward (2005).

The design of the bioscrubber mostly focuses on the removal of one chemical group of compounds only or even removal of one compound only (Sheridan *et al.,* 2003; Singh *et al.,* 2005). The performance of the odour control methods are carried out by olfactometry and/or analytical methods, *e.g.* GC, GC-MS (Melse and Ogink, 2005).

In connection to the mini review mentioned above:

This study is a part of a larger research project called 'Absorption in water droplets of odours, ammonia and dust from livestock buildings' (Figure 3). In which, odours from livestock buildings will be treated using a bioscrubber to reduce the nuisance. Key odorants were investigated in this study (Table 2). They were chosen by the Danish Ministry of Food, Agriculture and Fisheries / Danish Institute of Agricultural Sciences / Department of Agricultural Engineering, who is a partner in the project. Key odorants were chosen to represent a variety of chemical groups and due to their contribution in the nuisance problem from livestock buildings, although they are in relatively low concentrations. Key odorants will be absorbed in the absorption unit and degraded in the bioreactor. The absorption column and the bioreactor will be designed by other partners and colleagues.

This study contributes to the analytical measurement of odorants. Its aim is to identify and quantify the selected key odorants. These identification and quantification might be carried out before and/or after the bioreactor (as shown in Figure 3). The goal is to have an idea about the odorants absorbed by water droplets and the efficiency of the bioreactor. The identification and quantification will be carried out using gas chromatography and electronic tongue (next chapter). Using electronic tongue for characterization of odorants in livestock buildings was not reported before.

The concentrations of the key odorants in the pig farms, and not in poultry or cattle farms, were investigated in this study. This was done because: (1) the pigs and their meat production have a high influence in the Danish economy (Lara *et al.,* 2002), (2) the ultimate goal of the project is to reduce the nuisance problem caused by livestock buildings, and the nuisance problem in the pig farm is higher than the poultry and cattle farms (Hartung, 1992; Jongerbreur *et al.,* 2003), (3) the concentrations (using analytical methods) of these odorants in pig farms had been reviewed (O'Neil and Philips, 1992; Schiffman *et al.,* 2001), and (4) the presence of all key odorants in the pig farm, which results in a complex mixture of odorants (Table 2), *e.g.* sulphides, phenols and indoles.

Studying a complex mixture of odorants is an interesting research subject. However, characterization of key odorants in a pig farm will also imply the possibility of characterizing different odorants in poultry or cattle farms. This is because it is more comprehensive and difficult to characterize odorants in the pig farm.

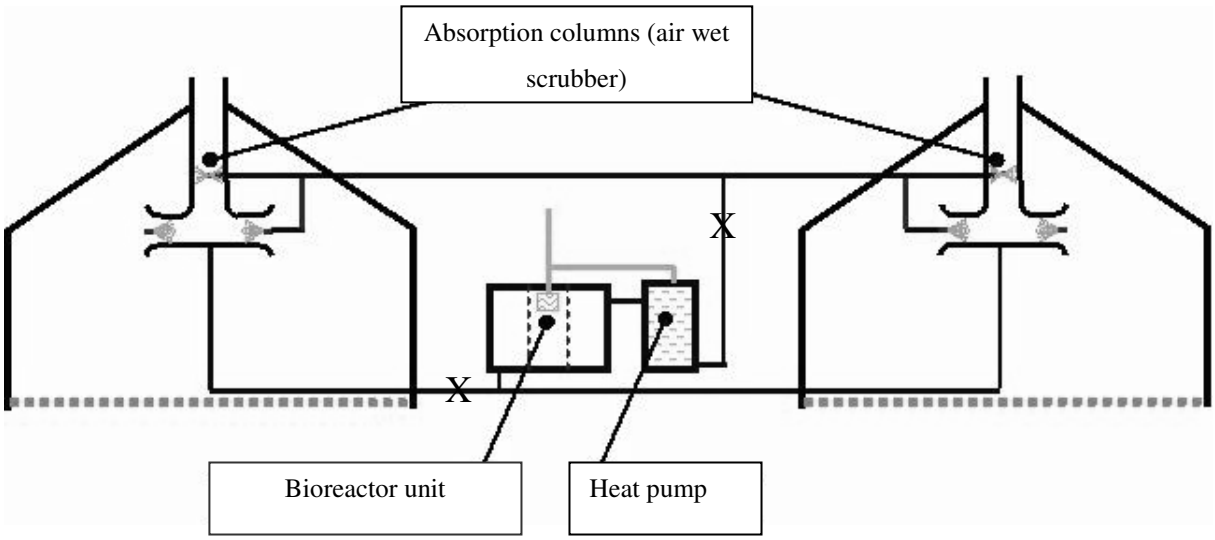

Figure 3. Sketch of project: 'Absorption in water droplets of odours, ammonia and dust from livestock buildings'. Odorants might be identified and quantifies before and/or after bioreactor (X: suggested positions that samples of water are measured). Heat pump is to control temperature of water, if needed (Source: DaNet, 2004)

Table 2. Key odorants investigated in this study

Group number	Group	Odorant number	Odorant	Chemical abstract service (CAS #)	Molecular formula	Number of citations in agricultural references according to presence of odorants in different livestock buildings (O'Neil *et al.,* 1992; Sunesson *et al.,* 2001)				Sensory and odour characteristics (Schiffman *et al.,* 2001)
						Pig	Poultry	Cattle	Sheep	
1	Sulphide	1	dimethyl sulphide	75-18-3	$(CH_3)_2S$	7	3	3	1	stench, decayed vegetables, putrid, disagreeable
2	Alcohol	2	1-butanol	71-36-3	$C_4H_{10}O$	5	0	1	0	irritant, fusel oil
3	VFAs	3	n-butyric acid	107-92-6	$C_4H_8O_2$	12	3	0	0	irritant, sweaty, rancid
		4	iso-valeric acid	503-74-2	$C_5H_{10}O_2$	8	0	0	0	rancid, cheese
4	Phenol	5	phenol (carbolic acid)	108-95-2	C_6H_6O	14	3	1	0	irritant
		6	4-methyl phenol (p-cresol)	106-44-5	C_7H_8O	16	4	1	0	irritant, phenolic
5	Indole	7	3-methyl indole (skatole)	83-34-1	C_9H_9N	13	4	0	0	stench, fecal odour, nauseating
6	Ammonia	8	ammonia	7664-41-7	NH_3	12	3	1	0	sharp, pungent

Reference of chemical properties: Syracuse Research Corporation (2005)

4. Electronic tongues (ETs)

Electronic tongues (ETs) have been developed quickly during the past few years. This is due to their large application potential and their promising alternative for many analytical methods. This chapter will discuss the different measurement principles, types, history, applications, advantages and limitations of ETs.

There are many analytical instruments that are used in biotechnology and industrial processes, *e.g.* mass spectrometry, gas chromatography, high performance liquid chromatography (HPLC), etc. Nevertheless, almost all of them are bulky, expensive, often time-consuming and require experienced operators (Gouma *et al.,* 2004; Legin *et al.,* 2004a).

Chemical sensors are an alternative. Stradiotto *et al.* (2003) defined a chemical sensor as: 'a device that provides continuous information about its environment. Ideally, a chemical sensor provides a certain type of response directly related to the quantity of a specific chemical species'. Chemical sensors mainly include electrochemical, optical and mass sensors. Use of electrochemical sensors is growing more rapidly than any other chemical sensors. This is due to their low cost, experimental simplicity and high detectability (Stradiotto *et al.,* 2003).

There are three main types of the electrochemical sensors: potentiometric (measure the voltage), voltammetric (measure the current), and conductometric (measure the resistance). The main basic electrochemical principles are potentiometry and voltammetry. Both of them required at least two electrodes, *i.e.* a working electrode and a reference electrode, and an electrolyte solution. The working electrode responds to the target molecular, and the reference electrode has a constant potential. The potentiometry method measures the potential difference of a charged membrane in contact with analytes, at no current. Different types of membrane materials have been used for this method. On the contrary, the voltammetry method measures the current at fixed potential. The current is related to the electron transfer reaction in the solution (oxidation and reduction) (Winquist *et al.,* 2004).

An electronic tongue (ET) is a chemical sensor. Most ETs are using electrochemical principles for measurements. The idea of the ET is based on using arrays that have cross-sensitive chemical sensors, combined with multivariate data analysis. The multivariate data analysis (explained in the next chapter) is important to analyse and understand the complicated signals produced by ET. Electronic noses (ENs) and ETs are based on the same concept, but ENs are used for gas analysis and ETs are used for liquid analysis. The ET is related to the human sense of taste, in the same way that the EN is related to olfaction. From this point, the ET was given this name (Legin *et al.,* 2004a; Winquist *et al.,* 2004). The term 'elec-

tronic tongue' was first suggested by an Italian-Russian research cooperation in a conference in Leuven, Belgium, in 1996 (Winquist *et al.,* 2004).

4.1. Advantages of ETs

The main advantages of ETs are: (1) relatively low cost, (2) simplicity of usage, (3) rapidity of measurement, (4) small size of sensors, which might be miniaturised, (5) measure directly in the solutions, (6) can be used in buffer media, (7) robust and better suited for measurements in industrial processes, (8) simultaneous determination of several components in the media, (9) the possibility of measuring ions, for which no ion-selective electrodes are known for measuring them, (10) measure in aqueous phase in which it will be able to provide information about ions and compounds that can be measured only in this phase (*e.g.* compounds having a low vapour pressure), (11) provide a larger amount of information about changes in the composition of the sample and this will provide a better understanding of the process, (12) suitable for some biological liquids, *e.g.* blood and blood plasma, where other sensors have some limitations, (13) safe and suitable for pharmaceutical application, especially in the absence of toxicological data for new medicine, (14) higher selectivity and lower detection limit than for a single sensor, and (15) require a small volume of sample (Legin *et al.,* 2004b; Legin *et al.,* 2004a; Soderstrom *et al.,* 2003a; Vlasov *et al.,* 2005; Vlasov and Legin, 1998; Winquist *et al.,* 2004).

4.2. Different types of ETs

The most important types of ETs that are mentioned in the literature until now are: (1) taste sensor, which was designed in Japan, (2) voltammetric electronic tongue, which was designed in Sweden, and (3) potentiometric electronic tongue which was designed in Russia. However, there are other scientific groups and types of ETs than mentioned.

4.2.1. Taste sensor / Japan

This was the first multi-sensor system, based on non-specific sensors for liquid solution. It used potentiometric method for measurement. It was introduced in Kyushu University / Japan, by Kiyoshi Toko and co-workers in 1990, and they named it 'taste sensor'. However, recently they referred to it as electronic tongue (Vlasov *et al.,* 2002). It is based on an array of eight different lipid/polymer membranes on a multi channel electrode. The voltage difference between the electrodes and a reference electrode (*i.e.* silver/silver chloride (Ag/AgCl)) is measured when the current is close to zero. However, different lipid materials are used depending on the object studied. Toko's group claimed that the taste sensor can mimic all the basic human biological tastes substances, *i.e.* sweetness, saltiness, sourness,

bitterness and 'umani'. Umani is the Japanese term for implying delicious taste (Toko, 1998). On the other hand, Vlasov *et al.* (2002) suggested that caution should be taken before generalizing that this device can mimic the human taste.

ET and taste sensor are somehow different. Taste sensor tries to mimic the operation of the human tongue to classify or identify the five different basic taste sensations. However, ET classifies or identifies the targeted solution without trying to mimic the human taste, and the results are not necessary compared with human sensation, but with other quality properties of the solution. Moreover, it was suggested that there is no need to compare ET signals with human sensory results in all applications, since this will limit the vision and applications of ETs (Winquist *et al.,* 2004).

The main area of the taste sensor applications is foodstuffs' quantification and recognition, *e.g.* beer, coffee, milk, mineral water and tomato juice (Toko, 1998). The taste sensor has been commercialized on the market and used in different industrial applications (Winquist *et al.,* 2004).

4.2.2. Voltammetric ET / Sweden

The voltammetric ET was firstly described by Fredrik Winquist and co-workers in Linkoping University / Sweden in 1997. It consisted of a reference electrode (Ag/AgCl), auxiliary electrode made of stainless steel and an array of two working electrodes constructed from two different noble metals, *i.e.* gold (Au) and platinum (Pt) (Winquist *et al.,* 1997). In the recent days, and after many modifications, the ET consists of reference electrode, auxiliary electrode and an array of six working electrodes, *i.e.* gold (Au), iridium (Ir), platinum (Pt), palladium (Pd), rhenium (Re) and rhodium (Rh). Nevertheless, the number of the electrodes used is depending upon the applications (Legin *et al.,* 2002b).

In this type of ET, current due to an electrochemical reaction from the redox active compounds (oxidation or reduction) present in the solution, is passed between the working electrode and the auxiliary electrode. At the same time, the applied potential to the working electrode is referred to a reference electrode of a constant potential. The current will depend on the type of working electrodes and the potential applied. The voltammetric method is robust and very sensitive. But in most cases, its selectivity is poor. The poor selectivity is due to that all the electrochemically compounds in the solution contribute to the measured current, due to their activity below the applied potential. One solution to overcome this problem is to apply pulse voltammetry, which it is more sensitive than constant potential. So, measurements in this type of ET are achieved using pulse voltammetry, in which volt-

age is applied to the metal electrodes in pulses of different amplitude, and the resulting current is collected for data analysis (Krantz-Rulcker *et al.,* 2001).

This type of ET has been used in many applications. Some of these applications are monitoring of milk deterioration due to the microbial growth when kept at room temperature (Winquist *et al.,* 1998), classification of six different microbial species (Soderstrom *et al.,* 2003b) and monitoring of drinking water production plants (Krantz-Rulcker *et al.,* 2001). Voltammetry ET is already commercialized on the market and utilized in several industrial processes (Winquist *et al.,* 2004). Some of the reported industrial applications are using of ET in house washing machines (Ivarsson *et al.,* 2005) and in dairy application (Winquist *et al.,* 2005).

4.2.3. Potentiometric ET / Russia

It was introduced by Andrey Legin and co-workers in St. Petersburg University / Russia. It was developed in 1996 as a part of an Italian-Russian research cooperation (Winquist *et al.,* 2004). The potentiometric ET is based on an array of ion-selective electrodes (ISEs). This ET has the advantages of being relatively cheap, rapidity of measurement and simplicity of usage. Moreover, ETs with an array of ISEs are the most wide spread type of ETs system until now (Stradiotto *et al.,* 2003; Vlasov *et al.,* 2005).

4.2.3.1. Ion selective electrodes (ISEs)

For an individual ISE, a linear relationship is assumed between ISE output (cell potential), and the logarithm of the activity (concentration) of the primary ion in the solution (Legin *et al.,* 2002b; Stradiotto *et al.,* 2003). The electrode should follow the Nerst equation, which is used for constructing a calibration curve:

$$E = E^o + \left(\frac{RT}{z_i F}\right) \times \ln a_i = E^o + Slope \times \ln a_i$$

where: E is the total potential (mV) developed between the sensing membrane and reference electrodes, E^o is the standard potential of the electrode, and it is characterized by the particular ISE/reference pair, as the intercept potential when the analyte activity is 1 M, R the gas constant (8.314 joules/(Kelvin × mole)), T is the temperature in Kelvin, z_i is the electrical charge of the primary ion (with sign), F is the Faraday constant (96500 coulombs/mole), and a_i is the activity of the primary ion, *i.e.* concentration. The term RT/z_iF is known as the response slope, which is the sensitivity of an ISE in the absence of interfering ions (Albert *et al.,* 2000; Legin *et al.,* 2002b).

In the case of presence of two compounds in the solution, *i.e.* primary and interfering ions, the Nikolsky-Eisenman equation can be applied (Vlasov *et al.,* 2005):

$$E = E^o + \left(\frac{RT}{z_i F}\right) \times \ln\left[a_i + \sum_j K_{ij}(a_j)^{z_i/z_j}\right] = E^o + Slope \times \ln\left[a_i + \sum_j K_{ij}(a_j)^{z_i/z_j}\right]$$

which is equivalent to:

$$E = E^o + 2.3 \times Slope \times \log\left[a_i + \sum_j K_{ij}(a_j)^{z_i/z_j}\right]$$

where: a_i and a_j are the activity of the primary and interfering ion, respectively, K_{ij} is the selectivity coefficient of the ISE to the primary ion *i* in the presence of the an interfering ion *j*, and the number 2.3 is the conversion factor from natural to base10 logarithm. This equation assumes a linear relationship between the sensor response, *E,* and the logarithm of the activity of the ions in the solution. However, this equation does not imply for a complex solution which contains many ions, *i.e.* more than two, which the electrodes are responsive for. Furthermore, the response of relation between *E* and logarithm of activity will be nonlinear (Legin *et al.,* 2002b; Vlasov *et al.,* 2005).

It can be noticed that selectivity and selectivity coefficient of the ISE are very important factors. The selectivity method refers to the degree to which ISE can determine particular analyte(s) in a complex mixture without interference from other components in the mixture. The selectivity coefficient is defined as the ability of an ISE to distinguish between a particular ion from the others. The smaller this value is, the greater is the effect of the primary ions. The selectivity coefficient between two compounds is determined by: (1) fixed interference method (FIM), and (2) separate solution method (SSM) (Buck and Lindner, 1994).

4.2.3.2. Multi-sensor array

The selectivity of ISEs in a complex mixture is a large problem. However, the use of low-selective sensors array together with the multivariate data analysis, *i.e.* ET, provides a solution to the selectivity problem. Usually the sensor array contains 10-30 sensors, depending on the application (Vlasov *et al.,* 2002). Figure 4 shows a schematic diagram of a potentiometric sensor array. An ET is defined as: 'an analytical instrument comprising an array of non-specific, low-selective, chemical sensors with high stability and cross-sensitivity to different species in solution, and an appropriate method of pattern recognition and/or multivariate calibration for data processing' (Vlasov *et al.,* 2005).

The terms high cross-sensitivity and low selectivity are important terms in ET, and the sensor array should comprise both of them. Cross-sensitivity is usually used to describe the multi-sensor systems. The cross-sensitivity means that the different sensors have to produce a stable response and/or to have the ability to respond in a reproducible way to different analytes present in a solution (Vlasov *et al.,* 2005). Three parameters were suggested for characterization of cross-sensitivity: average slope of sensors, reproducibility and non-selectivity. These parameters are evaluated on the basis of measurements in a set of individual components that are expected to present in the complex solution. Among the three parameters, the average slope of sensors is the most important factor for cross-sensitivity. The low selectivity of multi-sensor array, also named non-specific or low-selective, means that the sensors are not totally selective for one particular species in solution, but may well respond to diverse compounds in the solution (Legin *et al.,* 2002b; Vlasov *et al.,* 1997; Vlasov *et al.,* 2005).

Characterization of sensor array, *i.e.* cross-sensitivity parameters, selectivity and limit of detection, were investigated in some papers (Legin *et al.,* 1999b; Vlasov *et al.,* 1997). The cross-sensitive and low-selective sensors are important terms with respect to ET's performance. It was found that the array with cross-sensitive and low-selective sensors can significant improve selectivity and detection limit compared to discrete sensors. The detection limit of the sensor array was at least three times lower than that of the discrete sensor, under the same conditions (Legin *et al.,* 1999b).

4.2.3.3. Applications of potentiometric ET

Potentiometric ETs were used in many scientific applications. Some of these applications are:

* Characterization of different mineral water brands (Legin *et al.,* 1999a) and analysing the inorganic pollutants in the groundwater (Rudnitskaya *et al.,* 2001),
* Monitoring of fermentation process of *E. coli* (Turner *et al.,* 2003) and prediction of multi-components in the fermentation growth media (Legin *et al.,* 2004a),
* Prediction and assessment of the pharmaceutical products taste (Legin *et al.,* 2004b), and
* Classification of different apples varieties (Rudnitskaya *et al.,* 2006) and recognition of different liquids and water containing flesh food, *i.e.* fish (Legin *et al.,* 2002a).

4.3. Limitations and defects of ETs

The ETs are new technique. They were known since the beginning of the nineties of this century, *i.e.* 10-15 years ago. Naturally, there are some technical limitations and defects associated with their measurement principles and applications, due to their novelty and sensitivity. In addition, ETs have not yet reached their full potential to provide information and applied outside the laboratory (Soderstrom *et al.,* 2003a; Vlasov *et al.,* 2002). The researchers should be aware that the analytical capability of ETs should neither be overestimated (Winquist *et al.,* 2004) nor underestimated (Vlasov *et al.,* 2002). More research and applications related to ETs should be addressed.

The Japanese potentiometric taste sensor (*i.e*. ET) has a number of defects: (1) sensing mechanism is not clear and need more investigation, especially because the sensors are not ISEs, and (2) the potentiometric technique measures only ions (charged species) present in the solutions (Vlasov *et al.,* 2002; Winquist *et al.,* 2004).

The defects of the Swedish voltammetric ET are: (1) the differences between electrochemical reactions on the different sensor array, *i.e.* noble metals, are not clear and need more examination, and (2) the choice of the step size of the pulse voltammetry applied to the electrodes during measurement, are not fully explained (Vlasov *et al.,* 2002).

The defects of the Russian potentiometric ET are: (1) measurement of only ions in the solution, and (2) drift of the sensors (Holmberg *et al.,* 2004; Winquist *et al.,* 2004). Regarding the first point, Legin *et al.* (1999a) suggested that the responses of ET can be based on ionic, redox or molecular interaction at the membrane/solution interface. Furthermore, they recently stated that the ET mainly responds to ions (Soderstrom *et al.,* 2005). For the second point, *i.e.* drift, they suggested that drift is much related to the nature of the solution measured, and it can be overcome by using flow injection analyses or washing the electrodes between measurements until they reach their initial potential readings (Holmberg *et al.,* 2004).

Different versions of ET can show different analytical characteristics of the same samples under test. This is because the characteristics of ET, *i.e.* limit of detection, selectivity and cross-sensitivity, depend on the composition, design and the sensing materials used in the sensor array (Holmberg *et al.,* 2004; Vlasov *et al.,* 2005). Merging the data of different ETs for the same tested samples will give more information of the samples. Russian and Swedish ETs were used for recognition of four molds and one yeast. It was found that the merged data from both ETs improved discrimination of the samples in selected cases, despite that

both ETs alone can discriminate between different species (Soderstrom *et al.,* 2005). The Swedish and the Japanese ETs were used for classification of different tea and detergent samples. Extra information were obtained by combining data of the two sensor systems, despite that each ET is able to classify the samples separately (Ivarsson *et al.,* 2001). Also, combination of ET and EN data can help in improving the classification properties, *e.g.* fruit samples (Winquist *et al.,* 1999).

4.4. Perspective of ETs

Despite that the ETs are still in their 'infancy', the expectations of increased commercial and research interest towards them in the modern chemical sensor sector is high, especially after their successful applications in research laboratories and some real life applications. This interest is due to the need for advanced detection devices for many applications, *e.g.* health services, environmental technology and quality control. It is expected that sensors will play an essential role in human welfare in the future. The miniaturized sensors will contribute a lot to this point. The future is expected to be full of inexpensive, easy to handle and miniaturized sensors for our daily life (Gouma *et al.,* 2004; Vlasov *et al.,* 2002).

Vojinovic *et al*. (2006) reviewed many methods related to real time bioprocess monitoring, *e.g.* optical methods, nuclear magnetic resonance and ETs. They concluded that ETs are a promising new tool in bioprocess control, despite that they have not yet been used for *in situ* bioprocess monitoring. Moreover, they recommended further research and applications in ET area.

In connection to the mini review mentioned above:

A custom made prototype ET was purchased from Analytical Systems, Ltd., St. Petersburg – Russia. It consists of 14 potentiometric electrodes (*i.e.* sensors). Eleven polymer (PVC) plasticized membrane electrodes containing different active substances, two chalcogenide glass electrodes and one wire electrode. ET was designed to have a cross-sensitivity for the selected key odorants investigated in this study. Figure 4 shows a schematic diagram of the ET used.

The compounds used in the experiments are shown in Table 2. Different test mixtures of key odorants (*i.e.* solutions) were used in the experiments. However, dimethyl sulphide and 1-butanol were not included in the solutions. For dimethyl sulphide:

- ET has no response in the range of interest,
- Hard to wash the electrodes when dimethyl sulphide is included in the solution,

- No stable signal when dimethyl sulphide is included in the solution, and
- Constant drift of all electrodes reading, which indicated a possibility of damaging electrode membrane.

For 1-butanol, there was no-reproducibility of the electrode signals, *i.e.* signal jumping, when a solution contains 1-butanol.

Potentiometric measurements, *i.e.* potential difference between each electrode of the array and one common reference electrode, were performed using a high-input impedance multichannel voltmeter versus conventional Ag/AgCl reference electrode. A pH glass electrode was also included in the sensor array. The sensor array was connected to a computer for data acquisition. The data were deconvoluted using multivariate data analysis (next chapter).

The pH electrode was used to monitor the pH levels in the solutions during measurements. Maintaining the pH level at a constant value is important, to prove that ET does not only measure the pH, but the other compounds in the solutions.

In this study, ET was used for:

1. Studying the possibility of identifying and/or quantifying key odorants,
2. Classifying different test mixtures of key odorants, and
3. Simplifying the construction of the array and the data analysis by decreasing the number of electrodes, from the maximum number, *i.e.* 14 electrodes, to fewer electrodes. However, the selected electrodes should be sufficient for modelling key odorants and classifying different test mixtures of key odorants.

Papers number 2 and 3 discussed these issues.

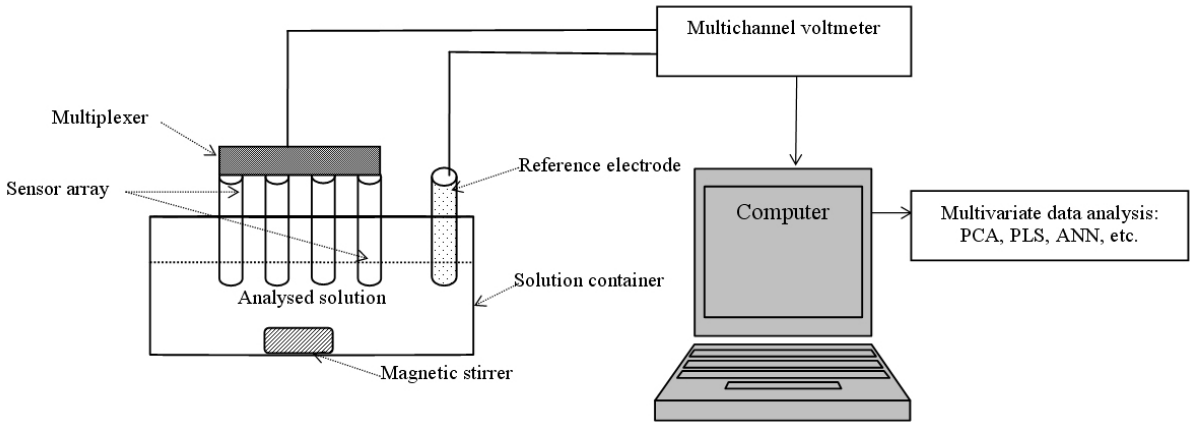

Figure 4. Schematic diagram of potentiometric electronic tongue

5. Multivariate data analysis (MVDA)

Multivariate data analysis (MVDA) or chemometrics is an important technique in different sciences. It simplifies the interpretation of the data and gives an idea about the quality parameters in concern. Moreover, it is the key for any sensor array development. In our study, ET produces complicated signals that might be solved using MVDA. The latter includes many techniques: linear (PCA, PLS, PLS-DA and SIMCA) or non-linear (ANNs, which contains many types, such as SOM and BPNN).

Many parameters are obtained during measurements and sensors monitoring of different processes. Methods to simplify the interpretation of large data sets are required. Multivariate data analysis (MVDA) or chemometrics is used to simplify the extraction of relevant information. Wold (1995) defined chemometrics as: 'how to get chemically relevant information out of measured chemical data, how to represent and display this information, and how to get such information into data'. Another reason for MVDA to become a powerful tool in different processes is due to the change in the philosophy of handling information in measurement technology. Quality parameters of the sample; *e.g.* condition, expected taste and state of process; rather than the quantitative chemical analysis of specific compounds, is desirable in many cases (Winquist *et al.,* 2004).

MVDA is used for ETs signal processing. MVDA extract the information from the complicated signals that ETs produce. The signals (pattern) contain information about different compounds and other features in the complex media. Different MVDA techniques are employed to extract these information. The applied techniques are depending on two factors: (1) the structure of the data, and (2) the goal of the research. For point no. 1, the structure of data refers to the linear or non-linear relation between the sensors response (independent variables, or predictors) and the concentration of the compounds (dependent variables) (Vlasov *et al.,* 2005). The non-linearity response of the sensors results from the interferences between ions in the media (Legin *et al.,* 2004a). For point no. 2, the ETs are applied for two tasks: qualitative (identification, classification and clustering) and quantitative (prediction) analysis. Qualitative analysis is mainly performed with principal component analysis (PCA), partial least squares-discrimination analysis (PLS-DA), soft independent modelling of class analogy (SIMCA), or self organizing map (SOM) (*i.e.* Kohonen net). Quantitative analysis is mainly performed with partial least squares (PLS) or back propagation neural network (BPNN) (Vlasov *et al.,* 2005). Table 3 summarizes the most used MVDA techniques for ET data processing, and some characteristics of these techniques. MVDA, including pattern recognition and calibration methods, is reviewed in many papers (Burns and Whitesides, 1993; Despagne and Massart, 1998; Jurs *et al.,* 2000; Pravdova *et al.,* 2002; Richards *et al.,* 2002; Svozil *et al.,* 1997).

Table 3. Multivariate data analysis techniques that mostly used for electronic tongue signal processing, and some characteristics of these techniques

Aim	*Method*	*Linear*	*Supervised*	*Advantages*	*Drawbacks*
1. Pattern recognition and classification	PCA	Yes	No	- Easy to understand and interpret (score plot, loading plot, etc.)	- Sensitive to drift in the data
	SOM	No	No	- Two dimensions of the data from any dimensionality	- Works as a black box (*i.e.* it is an ANN technique)
				- A promising method for data fitting and clustering for non-linearity cases	- Less commonly used than PCA
				- Used for data compression, while preserving their content	- Reduces the data to two dimensions only, while PCA expressed the reduced data to higher dimensions (*i.e.* different number of PCs)
	PLS-DA	Yes	Yes	- Work reliably when each class is 'tight' and occupies a small and separate volume in the X-space	- The number of modelled classes must not be high, *i.e.* classes are between 2-4
				- All measured variables play the same role with respect to the class assignment	
				- PLS components try to find a proper compromise between two goals: describing the set of explanatory variables and predicting the response ones	
	SIMCA	Yes	Yes	- Can be used when the classes are not 'tight', often due to the lack of homogeneity and similarity between non-tight classes	- Sensitive to the quality of the data used to generate the principal component models
				- Can work when the number of classes even exceed 4 classes	- Need more time, caution and experience than PLS-DA to build the classification models
				- Can be used to categorize samples that are not members of any class	
				- Unknown sample is only assigned to the class for which, it has a high probability to belong to	
				- Can work with few samples per classes, *e.g.* 10 samples per class	
				- Can work with a few numbers of variables (often the number of measurement variables exceeds the number of samples in chemical studies)	

Continue →

Table 3: Continued

Aim	*Method*	*Linear*	*Supervised*	*Advantages*	*Drawback*
2. Calibration	PLS	Yes	Yes	- Small calibration data set might be handled (cross validation is used in this case) - Statistical description of the results - Ability to analyse noisy, collinear, large amount of variables and missing values in X and Y matrices - Non-linearity can be solved, to some extent, by transformation of variables or including extra PCs (latent variables) to the model - There are non-linear PLS softwares available	- Sensitive to drift - Difficulty in interpreting the latent variables and the loading of the independent variables in very complex data - A lot of samples is needed for good models
	BPNN	No	Yes	- Easily deals with non-linear data - Flexible in terms of working in linear and non-linear models - High tolerance to data containing noise due to distributed processing within the network - Learning and underlying the relations between input and output without assistance of the user - The BPNN algorithm is used for solving both prediction and classification problems	- Works as black box (*i.e.* it is an ANN technique) - Good ANN models are always more time consuming than PLS models to produce - Experience for building models is needed - Lack some important features as model diagnostic tools - Lack of clear rules or fixed guidelines for optimal ANN architecture design. The optimal architecture is highly problem dependent and is sometimes found by trial and error

Sources:

Basheer and Hajmeer (2000); CAMO (2006); Dieterle *et al.* (2004); Eriksson *et al.* (2001); Legin *et al.* (2002b); NeuralWare (2003); Richards *et al.* (2002); Vlasov *et al.* (2005); Wold *et al.* (2001)

In the coming pages, we will mainly focus on PCA, PLS and ANN techniques, since they were used in this study.

5.1. Principal component analysis (PCA)

PCA is a famous method for processing multidimensional data in chemical applications. It is an unsupervised data reduction method. 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 decomposes into scores (which describes the relation between samples) and loadings (which describes the relation between variables). The principal components (PCs), also called latent factors (variables), are determined on the basis of the maximum variance criterion, and they are orthogonal. The first PC contains the most of the variance of the data. PCA results and graphs are rather easy to understand and interpret (Legin *et al.,* 1999a; Pravdova *et al.,* 2002).

A mathematical PCA model expresses the original data, X, in terms of scores and loadings is written as:

$$X = TP^T + E$$

where T is the score matrix, P^T is the transposed loading matrix and E is the residual matrix (noise). There are many algorithms for evaluating T and P matrices. The most common algorithms are non-linear iterative partial least squares (NIPALS) and singular value decomposition (SVD) (Richards *et al.,* 2002). However, the deep understanding of these algorithms is beyond the scope of this study. MVDA softwares handle the analysis using one of these algorithms.

Before performing PCA, each variable in the X matrix should be autoscaled (centred and divided by the standard deviation). The autoscaled data will have a mean of zero and unit variance for each column, *i.e.* a variable or a sensor signal. Autoscaling will guarantee transformation of the data to the origin, all the variables have the same variance and no dominant variables are presented (Wold *et al.,* 2001). Occasionally, only one part of autoscaling is carried out, *i.e.* centring or dividing by the standard deviation. This depends on the nature of the data handled, and it should be investigated during data analysis.

5.2. Partial least squares (PLS)

PCA and PLS are the so-called projection methods that allow one to efficiently reduce the number of original variables and reject noise (Legin *et al.,* 2004b). PLS regression is used to

correlate data in a *X* matrix (the independent variables) to a *Y* matrix (the dependent variables) in a linear way, by simultaneously finding latent structure (latent variables) in both matrices. In general, two PCA models are performed on *X* and *Y*, but not independently of each other. The inner relation is used to connect both models. In this way, mostly the information (variance) in *X* related to the phenomena of interest *Y* is extracted. Mathematically, *X* and *Y* are composed to:

$$X = TP^T + E$$

$$Y = UQ^T + F$$

where *T* and *U* are the score matrices, *E* and *F* are residual matrices and P^T and Q^T are the transposed loading matrices for *X* and *Y* matrices, respectively. A linear model is assumed to relate the score matrices *T* and *U,* with *H* as a residual matrix and *B* as a diagonal matrix that contains:

$$U = TB + H$$

B is the matrix containing the regression coefficients in the inner relation (Dieterle *et al.,* 2004; Richards *et al.,* 2002).

There are two types of PLS regressions. PLS-1, which uses just one *Y*-variable, and PLS-2, which uses more than one *Y*-variable. It is suggested that PLS-1 models often give better results than PLS-2 (Dieterle *et al.,* 2004). PLS has the ability to analyse noisy, collinear and missing values in both matrices. Moreover, PLS can handle, to some extend, the non-linearity problem, by mathematical transformation of the variables or including extra latent variables (PCs) in the model. However, there is non-linear PLS techniques available in some softwares (Pravdova *et al.,* 2002; Richards *et al.,* 2002; Wold *et al.,* 2001). MVDA softwares show the results of PLS in graphs (*e.g.* scores, loadings and predicted *vs.* measured) which make it easier to comprehend the results.

Partial least squares-discrimination analysis (PLS-DA) is used for classification. In PLS-DA coded dependent *Y*-variables (*i.e.* dummy variable) are employed. The number of dependent variables *Y* is equal to the number of the classes. Values of either 1 for samples belonging to the class, or -1 (some use zero) for samples not belonging to the class are assigned. Then PLS-1 or PLS-2 are used (Legin *et al.,* 2004b).

5.3. Artificial neural network (ANN)

Artificial neural networks (ANNs) are a part of artificial intelligence. They are algorithms simulating the biological neuronal system. They are capable of learning both linear and non-linear systems. ANNs are used for both classification and regression methods.

A feed forward ANN architecture (also known as multi-layer perceptrons (MLP)) consists of nodes (known as neurons) and weights associated to nodes. The nodes are arranged in layers. Each neuron in a layer receives the same inputs, x. The sum of each input multiplies by a weight, w, plus a bias, b, is then passed through a transfer function, f, to give a result t for that neural for the given input. This equation explains the process:

$$t = f\left(b + \sum_{i=1}^{n} w_i x_i \right)$$

The transfer functions might be linear or non-linear (*e.g.* sigmoid and hyperbolic tangent). Weight can be calculated during the training process, then the whole network is applied to samples (Copper, 2004; Pravdova *et al.,* 2002; Richards *et al.,* 2002).

There are many popular types of ANNs. One of the most famous and most widely used is back-propagation neural network (BPNN). It consists of three layers. They are: (1) an input layer, having nodes representing input variables for the model, (2) an output layer, with nodes having the dependent variables, and (3) one or more hidden layers, containing nodes trying to capture the non-linearity in the data. However, it is possible not to have a hidden layer, if the data structure showed that.

The data in BPNN are fed forward into the network. On the other hand, its name is back propagation neural network. This is because in this network the error computed at the output side is propagated backward, *i.e.* from the output layer to the hidden layer and finally to the input layer. During that process the weights of the interconnections are changing to get the minimum error between the input and the output targets. BPNNs are used for calibration and classification problems (Basheer and Hajmeer, 2000; NeuralWare, 2003; Richards *et al.,* 2002).

ANNs different techniques are often referred to as ‘black boxes’. This expression was given due to their incapability to explain, in a comprehensible way, the process through which a given answer was made (Basheer and Hajmeer, 2000). However, many authors argue about this point. Despangne and Massart (1998) stated that this limitation is not peculiar to ANNs only. ANNs work, in terms of prediction, as good as any other techniques. Copper (2004) suggested that with new ANNs software tools, *e.g.* sensitivity analysis, the relation between input and output variables and the factors effecting the result can be explained.

Generally, ANNs proved to give the best results for sensor array signals processing (Stefan *et al.,* 1999). With respect to the ET signals, in many experiments, quantitative results handled by PLS and BPNN, are not identical but very similar. BPNN can improve the non-linearity in the data, but it lacks some important features as visualization tools and clear rules about building models. However, PLS and/or BPNN are used for ET signal processing (Vlasov *et al.,* 2005).

In connection to the mini review mentioned above:

ET was used in this study for identification and/or quantification of key odorants that are present in livestock buildings. ET signals produced in the experiments were handled by two softwares:

1. The Unscrambler (v. 9.2, Camo, Oslo, Norway): which was used for PCA, PLS and PLS-DA, and
2. 'Predict' (v. 3.13, NeuralWare, Pittsburgh, USA): which was used for BPNN.

The 'Predict' software was employed because there were non-linear relations between the response of the sensors and the concentrations of the compounds (Vlasov *et al.,* 2005).

Each calibration model was initially checked by PLS linear technique, and depending on the non-linearity degree, BPNN might be used. The non-linearity between *X* (independent variables) and *Y* (dependent variables) matrices can be checked by many ways using Un-scrambler software. Some of these ways are:

- *X*-*Y* relation outliers plot in PLS (*i.e.* T-U score plot): might be used for non-linearity and also for detection of outliers,
- *Y*-residual plot,
- A 2-dimensional scatter plot of an array of one sensor response (*X*) and a concentration array of a compound (*Y*). This method is for an individual sensor and just to get an idea about linearity, and
- Normal probability plot of any sensor in *X* is valid for non-linearity checking unless *Y* is prepared in "equal" interval.

The articles attached to this thesis have all the results related to the experiments and data analysis using MVDA.

6. Summary and conclusion of the papers

Three papers are attached to this thesis. Figure 5 shows the schematic presentation of these papers.

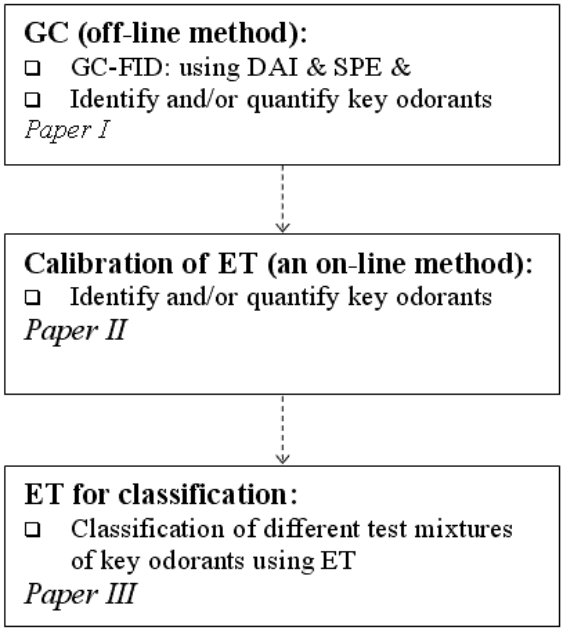

Figure 5. Schematic presentation of papers in this thesis

In the first paper, gas chromatography-flame ionisation detection (GC-FID) was utilized as an off-line method for characterization of key odorants in the absorption column (air wet scrubber). Direct aqueous injection (DAI) and solid phase extraction (SPE) methods were used before injection of key odorant into GC-FID. Both DAI and SPE methods were efficient for identification of odorants in the wet scrubber. However, DAI is the method of choice for quantification of odorants in air wet scrubbers, since it is simple, fast, requires small volumes, without pre-concentration and no derivatisation of the compounds is needed before injection into GC. Two odorants, *i.e.* phenol and 1-butanol, were quantified successfully using the DAI method. Their limit of detection (LOD) and limit of quantification (LOQ) were below literature values for odorants detection limits in livestock buildings.

The second paper discussed the possibility: (1) to identify and/or quantify key odorants occurring in livestock buildings using ET, and (2) to simplify the construction of the ET and the data analysis by decreasing the number of electrodes in ET as much as possible. Four test mixtures of key odorants were used for calibrating ET. Two test mixtures of key odorants at two different acidities, pH 6 and 8. It was seen that 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 quanti-

fied using six and four electrodes, respectively. Different multivariate data analysis techniques were used, *i.e.* PCA, PLS and BPNN. These techniques 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 reproducibility of ET because the standard deviation and relative standard deviation of measurements in triplicates decreased in comparison with the array comprising 14 electrodes.

In the third paper, the ET has successfully classified different test mixtures of key odorants. Eight electrodes were sufficient for classification using BPNN. The ET was able to distinguish between two test mixtures of key odorants at the same pH with classification rates in the range of 88 - 100%. Classification rates between the same test mixtures of key odorants at different pH were 100%. Different test mixtures of key odorants comprising a variety of the chemical groups at pH 6 were also successfully classified. The average classification rate (ACR) was 81%. The reproducibility of electrodes improved when the complexity of the test mixture was decreased.

The results from the second and third paper showed that nine electrodes in total were sufficient for identification, quantification and classification of all test mixtures of key odorants.

It is concluded that ET has a high potential and it is an obvious candidate as an on-line sensor for monitoring odorants in livestock buildings. Also, it might be used as an alarm system, for which there is a demand.

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Paper I

Nawaf Abu-Khalaf, Kim F. Haselmann, Jens Jørgen Lønsmann Iversen

Identification and quantification of odorants in an air wet scrubber using direct aqueous injection-gas chromatography (DAI-GC) and solid phase extraction-gas chromatography (SPE-GC)

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Identification and quantification of odorants in an air wet scrubber using direct aqueous injection-gas chromatography (DAI-GC) and solid phase extraction-gas chromatography (SPE-GC)

Nawaf Abu-Khalaf[1], Kim F. Haselmann[2], Jens Jørgen Lønsmann Iversen[1*]

[1]Department of Biochemistry and Molecular Biology, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark

[2]Department of Chemistry, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark

Abstract

Characterization of key odorants from an air wet scrubber is presented. The key odorants represent five chemical groups, *i.e.* sulphides, alcohols, volatile fatty acids (VFAs), phenols and indoles. Direct aqueous injection (DAI) and solid phase extraction (SPE) methods were used before gas chromatography-flame ionisation detection (GC-FID). The DAI and SPE methods were efficient for identification of odour compounds from the air wet scrubber. The SPE method had a high recovery. However, DAI showed a better linearity and a lower limit of detection (LOD) and quantification (LOQ) than the SPE method. The DAI method was preferred as it is cheaper, easier to handle, without sample preparation and highly applicable. At least two odorants, phenol and 1-butanol, were quantified successfully using the DAI method. Their LOD and LOQ were below literature values for odorants detection limits in livestock buildings.

Keywords: air wet scrubber, odorant compounds, direct aqueous injection (DAI), solid phase extraction (SPE)

∗ Corresponding author phone: +45 6550 2376; fax: +45 6550 2467, e-mail: jjli@bmb.sdu.dk

1. Introduction

Odour is an important environmental pollution issue [1]. An odour is defined as a sensation resulting from the reception of a stimulus by the olfactory sensory system [2, 3], whereas an odorant is the compound imparting an odour [4]. Odorant molecules emanating from different sources must be sufficiently volatile to arrive at the olfactory receptors in the nose. The molecular structures of odorants are very diverse, with a mass range up to approximately 300 Daltons [5].

The main sources of odours from animal production are livestock buildings, waste storage and land spreading of manure [6]. The emission of odours from livestock buildings contributes significantly to odour problems. This leads to environmental and health problems. It was found that neighbours of livestock buildings suffer from greater mood disturbance, negative emotions, an overall feeling of less vigour, more tension, depression, anger, fatigue and confusion compared with people living away from livestock buildings [7]. In addition, it was found that odours can also potentially affect memory [8].

There have been many attempts to reduce odours emission from livestock buildings, *i.e.* physical, chemical and biological. One of the biological method is to use bioscrubbing, which is considered as environmental friendly [9], and were used for air treatment in different industrial and agricultural activities [10, 11]. Bioscrubbers consist of two main parts, air wet scrubber (the absorption column) and a bioreactor. The air wet scrubber washes the polluted air stream and the bioreactor cleans the washing water coming from the air wet scrubber [12]. Therefore, the process of bioscrubbing is divided into two steps, the water soluble components in the gas (exhaust air) are transferred to the liquid phase in the air wet scrubber, and microorganisms metabolize different substances in the bioreactor. This results in the production of biomass, water and CO_2 [13].

Despite extensive information about bioscrubbers, they have not been successfully implemented in livestock buildings, mainly due to their high capital and operating costs, and due to the large volumes of air that must pass through the air scrubbers, *i.e.* high energy requirement [11]. A new type of livestock bioscrubber for treatment of larger volumes of air, without risk of high pressure loss over the scrubber column, has been introduced [14]. This bioscrubber consists of two separate units: an absorption column and a water purification module (a bioreactor), as shown in Figure 1. The absorption column is placed inside the ventilation chimney, where odour substances (odorants), ammonia and dust particles are absorbed by water droplets. Water droplets are introduced to the absorption column through water nozzles, who receive water recycled from the bioreactor, where the water is purified. The bioreactor is placed at floor level, and can supply cleansed water to several absorption

columns [14]. In the bioscrubber, it is necessary to characterize the mixture of odorants present in water, before and after the bioreactor for determination of the cleaning efficiency of the bioreactor.

Odours are measured analytically or sensorally. Analytical methods measure odorants, and sensory methods measure odours. The analytical methods characterise odorants in terms of their chemical or physical composition, with the most common measurement being odorant concentration. Analytical measurements have the advantages of objectivity, repeatability and accuracy. They are directly related to theoretical models with regard to odorant formation or emission and are more suited for formation, emission and dispersion models. However, a link between analytical and sensory measurements is needed [4]. The main barrier to that link is the effect of mixtures. It is often observed that a mixture of odorants will have a stronger odour than any of the component odorants alone, so that the effect of mixing will be additivity. However, the degree of additivity varies [15]. An analytical quantification of a mixture of odorants in water will be the first step in an effort to establish a comparison between the sensory and the analytical methods.

The full characterization of all the odorants present in a sample is an impossible task, as a large number of odorants are likely to be present at very low concentrations. Schiffman *et al.* [3] identified a total of 331 different odorants from livestock buildings. These compounds belong to different groups, *e.g.* alcohols, carbonyls, nitrogen-containing compounds, sulphur-containing compounds, ketones and aromatic organics among others. O'Neil and Philip [16] found 168 different compounds in livestock waste and in the air in livestock buildings, that contribute to odour, and 30 of them have detection thresholds of 0.001 mg/m^3 or less. Separation techniques followed by different identification methods are used. Purge and trap (P&T), solid phase micro extraction (SPME) and solvent extraction were used as a separation techniques for gas chromatography (GC) or gas chromatography-mass spectrometry (GC-MS) analysis [17-19].

Due to the huge numbers of odorants, seven key odorants, representing five groups, *i.e.* sulphides, alcohols, volatile fatty acids (VFAs), phenols and indoles, were chosen as key odorants in this study (Table 1).

The chemical and physical properties of the key odorant are shown in Table 1. The *pKa* for neutral 3-methyl indole (skatole) was not available in literature. The *pKa* for indole, *pKa* = 16.7, was used to give an idea about the dissociation of skatole [20]. The compounds investigated are polar or moderately polar. They are present in various livestock buildings and have an offensive odour [16]. The concentrations of these compounds in air were investigated by many researches. O'Neil and Philip [16] and Schiffman *et al.* [3] reviewed the odorant

detection thresholds in livestock buildings. These odorant detection thresholds of key odorants in air will be used in this study.

In the air wet scrubber, these compounds are present in the liquid phase rather than in the gas phase, and their concentrations are calculated using Henry's constant *(H)* (see Table 1), assuming an equilibrium between gas and liquid. 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). The dimensionless air-water partition coefficient (K_{AW}) is H/RT, and is the air to water concentration ratio at equilibrium [21]. The dimensionless air-water partition coefficient represents volatility of the compound. A compound with K_{AW} of 0.05 or larger is volatile, whereas those with a K_{AW} lower than 0.05 tend to occur in the water phase [22]. Almost all of the targeted compounds, except dimethyl sulphide, have a lower K_{AW} than 0.05 and they tend to occur in water. The detection thresholds of the targeted compounds in air and the equivalent odorant detection threshold in water are shown in Table 2.

In this work, the characterizations of key odorants were carried out using two methods, *i.e.* direct aqueous injection-gas chromatography (DAI-GC) and solid phase extraction-gas chromatography (SPE-GC).

DAI-GC was used both in the laboratory and in the field for the detection of fuel oxygenates, benzene, toluene, ethylbenzene and xylenes (BTEX) with very high accuracy [23], in the quantification of major volatile compounds and polyols in wine [24] and for quality control of water [25].

SPE could be regarded as a specific type of column chromatography, switching between full retention using *e.g.* water as mobile phase, and no retention using *e.g.* 100% organic solvent as mobile phase [26]. Different sorbents are available for different applications. Successful applications of SPE include the analysis of flavour compounds in milk, preservatives and sweeteners in soft drinks, colourants in alcoholic beverages, pesticides in water [27] and several medical examples [28-31].

The goal of this study is to characterize the key odorants in an air wet scrubber, according to their odorants detection threshold, using DAI-GC and SPE-GC and to compare the two methods with respect to the air wet scrubber application.

2. Experimental section

2.1. Sample preparation

2.1.1. Materials

All compounds were purchased from Sigma-Aldrich (Schnelldorf, Germany), phenol and 3-methyl indole (skatole) as solids. Skatole was dissolved in hot Millipore water [32]. Phenol was dissolved in Millipore water. Dimethyl sulphide, 1-butanol, n-butyric acid, iso-valeric acid and 4-methyl phenol have purity of 99%. Phenol and 3-methyl indole have purity of 99.5% and 98% respectively. Compounds were used without any further purification. Ten standard solutions were prepared in Millipore water with a concentration range between 10-5000 mg/m^3 for VFAs and 4-methyl phenol, between 5-2490 mg/m^3 for 3-methyl indole, between 15-7500 mg/m^3 for 1-butanol and phenol, and between 15-7537 mg/m^3 for dimethyl sulphide. The same standard solutions were used for the DAI and the SPE methods. Acetone (HPLC quality) was used as a solvent for SPE and Millipore water for DAI.

2.1.2. Direct Aqueous Injection (DAI)

Samples for DAI were acidified to approximately pH 2, by adding 0.2% (v/v) formic acid. Injection volumes of 0.5 and 1.0 µl were used in duplicates.

2.1.3. Solid Phase Extraction (SPE)

The analytes were extracted from the solution of odorants mixture using Strata-X polymeric SPE cartridges (Phenomenex, CA, USA), with 500 mg adsorbent and 3 ml reservoir volume. Strata-X is a modified styrene-divinylbenzene polymer suitable for a wide range of basic, neutral and acidic compounds [28, 29], and can separate trace amounts of chemical compounds from a complex solution [27].

A 10 ml aliquot was taken from each standard solution. The pH was adjusted to 2 by addition of concentrated HCl. Before sample loading, the SPE columns were conditioned by washing with 5 ml methanol, and equilibrated with 5 ml of deionized water. Before the column dried, the samples were loaded onto the conditioned column with the aid of a vacuum manifold. The mean flow rate was about 2.5 ml min^{-1}. The analytes were eluted with 2 ml of a mixture of acetone and methylene chloride, CH_2Cl_2 (1:1 v/v). The eluted solvents were collected for analysis. Duplicates of SPE samples were carried out.

The recovery and breakthrough of SPE columns were investigated before using the Strata-X column to make calibration curves. The solutions that were eluted to investigate recovery and breakthrough, had at least threefold higher concentration than the maximum concentration of each compound, that were used for the calibration curves in the SPE experiment. Recovery of the SPE columns was evaluated through double elutions, *i.e.* after finishing elution from the

column, another elution was done within the same column, and injected into the GC in duplicate. Breakthrough of the column is the point, where the SPE sorbent becomes saturated and unable to retain additional analytes. Breakthrough was evaluated by using two SPE columns, *i.e.* collecting the eluted sample from the first SPE column, load it once more on another SPE column and then elute the collected samples from both columns into the GC. Same procedure of elution was followed for both columns.

2.2. Gas Chromatography (GC)

An Agilent gas chromatograph (HP 6890), containing a capillary column (Zebron ZB-Wax, Phenomenex, CA, USA) 30 m long × 320 µm inner diameter × 0.25 µm nominal film thickness, with a cool-on-column injector (COC) and flame ionisation detection (FID), with a maximum temperature of 300°C, was used. A 5 m deactivated precolumn of the same diameter was used before the analytical column. The temperature program was: 35°C for 5 min, then increased to 225°C at 10°C/min and kept at this temperature for 15 min. The total run time was 39 min. According to our preliminary experiments with DAI, a modified temperature program was required for improved identification of 1-butanol: 35°C for 5 min, then increased to 120°C at 30°C/min, then increased to 225°C at 10°C/min and kept at this temperature for 10 min. The total run time was 28.33 min.

The injection volume was 1 µl in the case of DAI and SPE. For 1-butanol using DAI, two injection volumes (1.0 µl and 0.5 µl) of odorants were tested. Hydrogen was carrier gas with a constant flow rate of 2.5 ml/min, corresponding to an initial head pressure of 43.7 kPa. Samples were injected using an auto sampler (HP 6890 injector) with a slow plunger speed. Manual integrations were done for small peaks in the chromatogram.

The limit of detection (LOD) and limit of quantification (LOQ) were determined from the calibration curves according to Miller and Miller [33], *i.e.* LOD is three times the standard deviation of the noise / slope ratio of the calibration curve, and LOQ is ten times the standard deviation of the noise / slope ratio of the calibration curve.

3. Results and discussion

3.1. Direct aqueous injection (DAI)

Injection of samples containing a high amount of water into a chromatographic column will affect the efficiency of the column, lead to degradation of the stationary phase, and create active sites resulting in low peak resolution, poor reproducibility and shortened column life [24]. However, the structure of the stationary phase in capillary columns has been improved [34]. The Wax stationary phase is thermally stable, inert and has high endurance with repeated injection of aqueous samples [35]. The Wax columns are recommended for many

separations, including alcohols and aroma compounds [24]. However, there is still a need for frequent system maintenance to avoid troublesome effects, *e.g.* peak tailing, reduced recovery and sensitivity, caused by water. The precolumn is used in the GC to reduce these troublesome effects. It is frequently shortened, and is recommended to replace it after about 1000 injections [23].

Water samples spiked with the key odorants, were injected into the Wax column in the GC. Samples were acidified to approximately pH 2 by adding 0.2% (v/v) formic acid, since it was impossible to identify the VFAs in the chromatogram without acidifying the samples.

3.1.1. Identification and quantification of compounds

The chromatogram of the DAI is shown in Figure 2. The injection volume has an effect on the peak shape, especially for alcohols [23]. Therefore two injection volumes, *i.e.* 1.0 µl and 0.5 µl, of odorant mixture were investigated. The goal was to find the volume that produces the best peak shape for 1-butanol. The characterization of 1-butanol mentioned in Table 3 was determined based on two temperature programs, and two injection volumes. A volume of 1.0 µl, using the temperature program that was used for all the other compounds and two injection volumes of 1.0 µl and 0.5 µl, using the temperature program only used for 1-butanol. Generally, the peak shape of the 0.5 µl injection volume was only slightly better than that of the 1.0 µl sample (Figure 3). The latter will therefore be used as the injection volume in further experiments, especially with samples containing low concentrations. This is in agreement with Zwank *et al.* [23] who recommended an injection volume of 1 µl for alcohols.

The performance of the DAI-GC is shown in Table 3. The adjusted retention time, which is the difference between the dead time and the retention time for a compound, was used to identify the odorants. The dead time is the time required for the mobile phase to reach the detector [36]. The range of the standard deviation in the adjusted retention time was between 0.00 - 0.16 minute; 1-butanol had the highest standard deviation that reflects the difficulty in assigning the peak position of broad peaks.

The calibrations curves showed linearity with good correlation coefficients (R^2= 0.99) for the range specified. The LOD was used to assess the possibility of identifying odorants in low concentrations. Four compounds: 1-butanol, n-butyric acid, iso-valeric acid and phenol, had limit of detections which were below the equivalent odorant detection threshold reported by Schiffman *et al.* [3]. Two compounds: 1-butanol and phenol, had limit of detections which were below the minimum equivalent odorant detection threshold reported by O'Neil and Philips [16]. Three of the compounds, *e.g.* n-butyric acid, iso-valeric acid and 4-methyl phenol, had limit of detections which were between the minimum and maximum equivalent odorant detection threshold reported by O'Neil and Philips [16].

The limit of quantifications of phenol and 1-butanol were below the equivalent odorant detection thresholds reported by both reviewers. While the n-butyric's LOQ was below the equivalent odorant detection threshold reported by Schiffman *et al.* [3], and was between the equivalent odorant detection thresholds reported by O'Neil and Philips [16].

These results suggest that at least two compounds, *i.e.* 1-butanol and phenol, had LOD and LOQ that were below the equivalent odorant detection threshold reported by both reviewers, and they were identified and quantified successfully. These two compounds can therefore be used as representatives of the key odorants to give an idea about the efficiency of the air wet scrubber and the biofilter, as illustrated in Figure 1. Most existing bioscrubber designs focus on the removal of one chemical group of compounds [12], or even removal of one compound only [37]. Therefore our method, which identifies and quantifies at least two compounds, will improve the characterization of the bioscrubber.

DAI is a fast and simple technique that only requires small volumes and no pre-concentration. Moreover, it requires no derivatisation of the compounds before injection into the GC. DAI has acceptable sensitivity and is comparable with other analytical methods [23]. In addition, compounds are quantified regardless of their boiling points, which is a limitation in some analytical methods [19]. The results of this study showed that DAI is a convenient method for identification and quantification of odorants in the air wet scrubber.

3.2. Solid Phase Extraction (SPE)

3.2.1. Recovery and breakthrough

Recovery and breakthrough results of the Strata-X column are shown in Table 4. A high recovery was obtained, with a mean > 99% and relative standard deviation (RDS) < 1%. The breakthrough was almost absent, with a mean of < 1%. These results were calculated omitting dimethyl sulphide. If dimethyl sulphide was included, the recovery and the breakthrough became > 89% and < 15% respectively. Dimethyl sulphide is at the boiling point limit and is unstable [38, 39], and therefore excluded in the analyses by some researchers [40]. However, the recovery and breakthrough results showed a high performance in comparison with other studies [28], and they are in agreement with Zhang *et al.* [31] and Coulibaly and Jeon [27] who stated that SPE provides high recovery and clean extracts. These results indicate that Strata-X columns have a good separation capacity for the compounds of interest, even though they have different chemical properties.

3.2.2. Identification and quantification of compounds

Figure 4 shows the chromatogram of odorants extracted by Strata-X column. It appears that the column was unable to identify and quantify the VFAs. However, it could identify them in the recovery and breakthrough experiment (Table 4). This is most likely explained by the

concentration, which was used in the recovery and breakthrough experiment. This concentration was at least threefold higher than the concentration used for making the calibration curves. This higher concentration allowed the VFAs to be available in sufficient amount in free acid forms, so they appeared in the chromatogram in the recovery and breakthrough experiment. Also, the VFAs in the calibration experiment might be lost during the SPE process.

The performance of the SPE is shown in Table 5. The range of the standard deviation in the adjusted retention time was between 0.1 – 0.11 minute. The highest standard deviation was for phenol. Nevertheless, the absolute retention time showed an acceptable identification of odorants. Calibration curves showed linearity with correlation coefficients of > 0.977, when omitting dimethyl sulphide.

It was noticed that the LOD and LOQ of two compounds, *i.e.* 1-butanol and phenol, were below the equivalent odorant detection threshold reported by Schiffman *et al.* [3]. One compound, phenol, had a LOD that was below the minimum equivalent odorant detection threshold reported by O'Neil and Philips [16]. Two of the compounds, *e.g.* 1-butanol and 4-methyl phenol, had limit of detections and limit of quantifications that were between the minimum and maximum equivalent odorant detection threshold reported by O'Neil and Philips [16]. Phenol was the only compound which had both LOD and LOQ that were below the equivalent odorant detection threshold reported by the two reviewers [3, 16]. It was noticed that the peak of 4-methyl phenol splits gradually into two peaks, when decreasing the quantity injected into the GC. This may be due to the use of acetone and methylene chloride as solvents.

The LOD and LOQ of odorants were higher when using SPE in comparison to DAI. This may be due to the complexity of the compounds and the loss of the volatile compounds during the process of extraction and concentration. Moreover, SPE is a sensitive sample preparation technique [27] and has more extraction steps than in the DAI. However, the LOD and LOQ of 3-methyl indole were almost the same in both methods. This indicates 3-methyl indole was the best compound to be quantified using SPE in our mixture.

From the results above, it is concluded that Strata-X can be used for identification of odorants in air wet scrubber.

4. Application and perspectives

A water sample was provided to our laboratory by a company, running an experimental air wet scrubber in a Danish farm. It is noteworthy that to have as many compounds as possible absorbed in water, the ratio between the water flow and gas flow should be at least equivalent to the dimensionless air-water partition coefficient *(K_{AW})* [12]. The pH of the sample was 7. It

was noticed that for acidifying the samples to approximately pH 2, more formic acid than used for the samples creating the calibration curves was needed. This may be due to the presence of compounds having buffer capacity in the water sample. The chromatogram of the spiked samples is shown in Figure 5. The spike recoveries are listed in Table 6. It is seen that the recoveries are near 100% for most of the compounds, and this proves that that DAI method is suitable for quantifying the key odorants.

There are huge variations in odorant concentrations and odorant detection thresholds for odorant compounds from livestock buildings. This is because the odorant concentration is related to many factors, *e.g.* environmental factors, dietary feed quality, measuring methods and sources of sample [41].

It is possible in one report to find an odorant detection threshold higher than a minimum concentration of the same odorant in another report or vice versa, *e.g.* for phenol, the odorant detection threshold is higher than the minimum concentration reported by two reviewers [3, 16]. Therefore we conclude that although the LOD and LOQ of some compounds in this study were higher than the minimum equivalent detection threshold, there is still a possibility that DAI method can identify or quantify these compounds, because of the huge variation in concentrations reported.

Our method might be used for quantification of targeted compounds in water samples. It can also be used as a reference measurement method for measurements of odorant concentrations in an air wet scrubber. A new method was recently described, the electronic tongue (ET) which has high potential as an on-line sensor for analysis of liquids from the air wet scrubber. ET is an analytical instrument containing an array of chemical sensors with partial specificity for different components in liquids and an appropriate pattern recognition or multivariate calibration tool for identification and quantification of even complex solutions [42]. ET was used in various applications, *e.g.* for quantitative analysis of mineral water and wine [43], for analysis of fermentation growth media [44], for pharmaceutical analytics [45] and monitoring of the quality of drinking water in a production plant [46]. It is possible to place a calibrated ET in the air wet scrubber to identify and quantify the odorants of interest on-line. On-going research is taken now place in our laboratory for this purpose.

In conclusion we find that DAI is a suitable method for identification and quantification of odorants with a good precision in the air wet scrubber. Two compounds: 1-butanol and phenol, have LOD and LOQ that are below the equivalent odorant detection threshold. DAI is the method of choice for quantification of odorants in air wet scrubbers, and DAI can check their efficiency for odour reduction. DAI is fast, simple, without pre-concentration, requires

small volumes only and no derivatisation of the compounds is needed before injection into GC. DAI can be used as a quality control method for the air wet scrubber design.

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Table 1. Chemical and physical properties of key odorants

Group	Odorant	Chemical abstract service (CAS #)	Molecular formula	Molecular mass ($g\ mol^{-1}$)	Solubility in H_2O 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 °C	Boiling point °C
Sulphides	dimethyl sulphide	75-18-3	$(CH_3)_2S$	62.13	22	35.1	1.61	502	0.92	-98.3	37.3
Alcohols	1-butanol	71-36-3	$C_4H_{10}O$	74.12	63.2	16.1	8.81×10^{-3}	6.7	0.88	-89.8	117.7
VFAs [a]	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
	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
Phenols	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-methyl phenol	106-44-5	C_7H_8O	108.14	21.5	10.3	1×10^{-3}	0.11	1.94	35.5	201.9
Indoles	3-methyl indole	83-34-1	C_9H_9N	131.18	0.498	≈ 16.7 [b]	2.13×10^{-3}	0.00555	2.60	97.5	266

Reference of properties: Syracuse Research Corporation (2005) [47]

[a] VFAs: volatile fatty acids

[b] This value is for indole [20]

Table 2: Detection threshold concentrations of key odorants in air and water

Odorant	Dimensionless air-water partition coefficient (K_{AW}) [c]	Odorant detection threshold in air [16] (mg/m^3)		Equivalent odorant detection threshold in water (mg/m^3) [d]		Odorant detection threshold in air [3] (mg/m^3)	Equivalent odorant detection threshold in water (mg/m^3) [e]
		Min.	Max.	Min.	Max		
dimethyl sulphide	6.58×10^{-2}	0.0003	0.16	5×10^{-3}	2.4	0.00589	895×10^{-4}
1-butanol	3.60×10^{-4}	0.158	42	439	12×10^{4}	1.51	41.9×10^{2}
n-butyric acid	2.19×10^{-5}	0.0004	42	2×10^{1}	1.9×10^{6}	0.0145	663
iso-valeric acid	3.40×10^{-5}	0.0002	0.0069	6	2.0×10^{2}	0.0105	308
phenol	1.36×10^{-5}	0.022	4	16×10^{2}	3×10^{5}	0.427	314×10^{2}
4-methyl phenol	4.09×10^{-5}	0.00005	0.024	1	5.9×10^{2}	0.00832	204
3-methyl indole	8.70×10^{-5}	0.00035	0.00078	4.0	9.0	0.00309	35.5

[c] $K_{AW} = H / RT$, where: R: gas constant = 0.0821 atm. l. / (mol. K), T: temperature in Kelvin = 273 + 25 = 298

$K_{AW} = H$ (atm. l. / mol) / 24.47

K_{AW} = Concentration in air (C_a) / Concentration in water (C_w) $\Rightarrow C_w = (24.47 \times C_a) / H$ (atm. l. /mol)

[d] C_w: Calculated according to concentration of targeted compounds in air reported by O'Neil and Philips [16]

[e] C_w: Calculated according to concentration of targeted compounds in air reported by Schiffman *et al.* [3]

Table 3. Performance data for DAI-GC method (n=2). Injection volume is 1µl unless other values are stated

Odorant	Calibration equation	Rectilinear range used in calibration curves (mg/m^3)	Correlation coefficient (R^2)	Adjusted retention time (mean ± SDev [h]) (min)	Limit of detection (LOD) (mg/m^3)	Limit of quantification (LOQ) (mg/m^3)
dimethyl sulphide	$y = 47 \times 10^{-4} x + 26 \times 10^{-2}$	150-7537	0.999	0.31 ± 0.00	181	602
1-butanol [f]	$y = 13 \times 10^{-3} x - 34 \times 10^{-4}$	150-7500	0.999	7.13 ± 0.16	95	315
1-butanol [g]	$y = 13 \times 10^{-3} x + 24 \times 10^{-2}$	150-7500	0.999	5.58 ± 0.14	151	503
1-butanol, 0.5 µl injection volume [g]	$y = 65 \times 10^{-4} x + 21 \times 10^{-2}$	150-7500	0.999	5.70 ± 0.07	209	697
n-butyric acid	$y = 84 \times 10^{-4} x - 38 \times 10^{-2}$	100-5000	0.999	13.71 ± 0.14	130	433
iso-valeric acid	$y = 94 \times 10^{-4} x + 39 \times 10^{-2}$	100-5000	0.999	14.08 ± 0.12	114	381
phenol	$y = 15 \times 10^{-3} x - 0.11 \times 10^{-1}$	150-7500	0.999	17.19 ± 0.03	158	527
4-methyl phenol	$y = 14 \times 10^{-3} x - 14 \times 10^{-1}$	100-5000	0.999	17.88 ± 0.02	219	730
3-methyl indole	$y = 17 \times 10^{-3} x - 60 \times 10^{-2}$	62-2490	0.999	21.30 ± 0.01	59	197

[f] The temperature program: 35°C for 5 min, then increased to 225°C at 10°C/min and kept at this temperature for 15 min

[g] The temperature program: 35°C for 5 min, then increased to 120°C at 30°C/min, then increased to 225°C at 10°C/min and kept at this temperature for 10 min

[h] SDev: Standard Deviation

Table 4. Recovery and breakthrough of Strata-X columns for key odorants (n=3)

Odorant	Recovery within the same column (%) [i]		Breakthrough (%) [k]
	(mean ± SDev)	RSD [j]	(mean ± SDev)
dimethyl sulphide	89.64 ± 4.54	5.06	15.19 ± 1.85
1-butanol	100 ± 0.00	0	0
n-butyric acid	100 ± 0.00	0	0
iso-valeric acid	99.85 ± 0.27	0.27	0
phenol	99.81 ± 0.14	0.14	0.07 ± 0.03
4-methyl phenol	99.28 ± 0.49	0.50	0.26 ± 0.05
3-methyl indole	99.15 ± 0.81	0.82	0.18 ± 0.04

[i] Recovery within same column = A1 / (A1 + A2), where:

A1: area under curve from first elution of column

A2: area under curve from second elution of same column

[j] RSD: Relative Standard Deviation, RSD = 100 × SDev / mean [33]

[k] Breakthrough = A3 / (A1 + A3), where:

A3: area under curve from collected solution in second column

A1: area under curve from first column

Table 5. Performance data for SPE-GC method (n=2)

Odorant	Calibration equation	Rectilinear range used in calibration curves (mg/m^3) [l]	Correlation coefficient (R^2)	Adjusted retention time (mean ± SDev) (min)	Limit of detection (LOD) (mg/m^3) [l]	Limit of quantification (LOQ) (mg/m^3) [l]
dimethyl sulphide	$y = 59 \times 10^{-4} x + 3.1 \times 10^{1}$	377-7537	0.985	0.30 ± 0.07	1380	4602
1-butanol	$y = 32 \times 10^{-4} x - 4.3 \times 10^{1}$	187-7500	0.998	6.31 ± 0.02	512	1706
n-butyric acid	nn [m]					
iso-valeric acid	nn [m]					
phenol	$y = 29 \times 10^{-3} x - 4.8 \times 10^{1}$	187-7500	0.997	17.21 ± 0.11	594	1980
4-methyl phenol	$y = 29 \times 10^{-3} x + 12 \times 10^{1}$	125-5000	0.997	17.84 ± 0.03	379	1263
3-methyl indole	$y = 32 \times 10^{-3} x - 45 \times 10^{-2}$	50-2490	0.999	21.14 ± 0.01	57	190

[l] Before re-concentration. Compounds were re-concentrated 5 times (10 ml eluted / 2 ml of elution solvent = 5 times) using Strata-X columns

[m] nn: not found

Table 6. Recovery of key odorants in water sample from air wet scrubber matrix (n=3)

Odorant	Recovery (%) (mean ± SDev)
dimethyl sulphide	98.9 ± 11.9
1-butanol	89.9 ± 1.4
n-butyric acid	132.5 ± 10.7
iso-valeric acid	300 ± 8.4
phenol	98.3 ± 2.7
4-methyl phenol	104.0 ± 5.2
3-methyl indole	83.5 ± 3.5

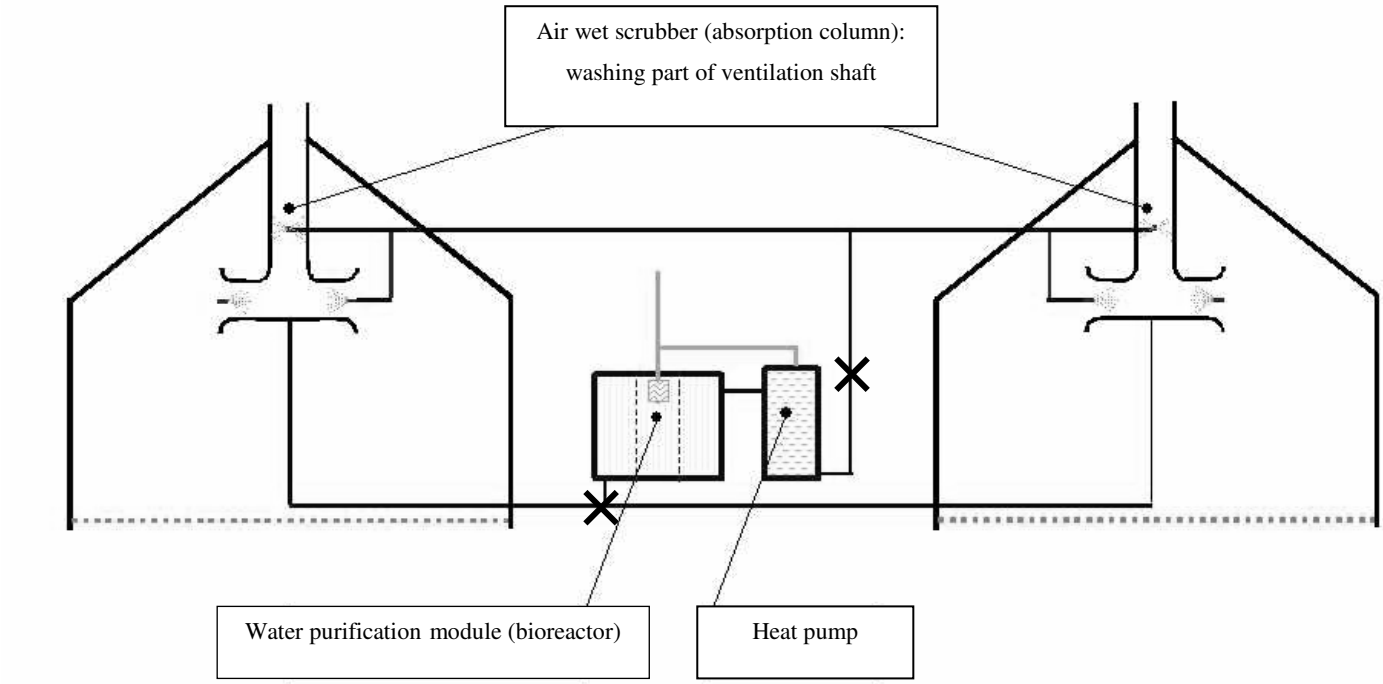

Figure 1. Schematic outline of livestock building bioscrubber (X: points at which samples containing mixture of odorants are measured). Heat pump is used to control temperature of water [14]

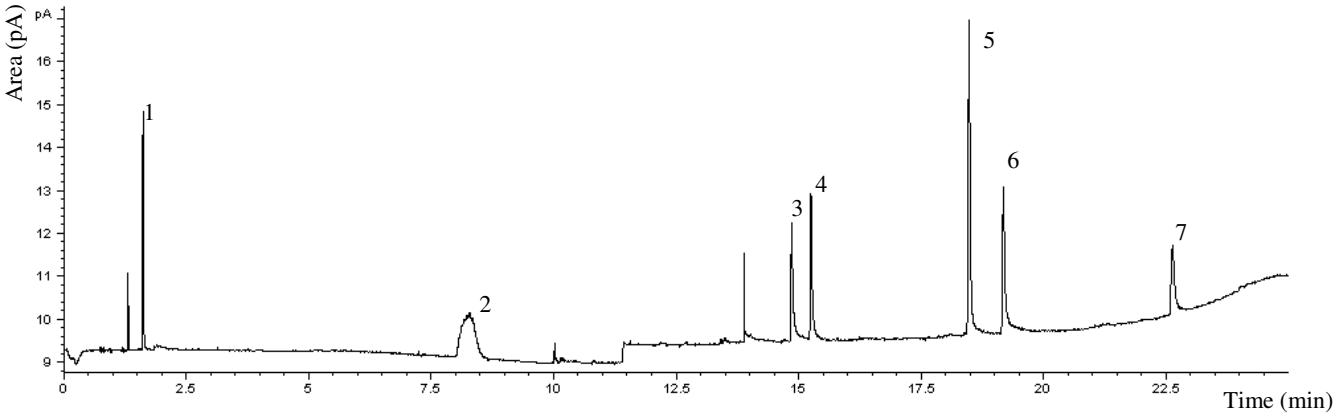

Figure 2. GC chromatogram of direct aqueous injection (DAI) water sample spiked with 1000 mg/m^3 volatile fatty acids (VFAs) and 4-methyl phenol, 498 mg/m^3 3-methyl indole, 1500 mg/m^3 1–butanol and phenol, and 1507 mg/m^3 dimethyl sulphide (DMS) (1: DMS, 2: 1-butanol, 3: n-butyric acid, 4: iso-valeric acid, 5: phenol, 6: 4-methyl phenol, 7: 3-methyl indole). Injection volume: 1.0 µl. Temperature program: 35^{o}C for 5 min, then increased to 225^{o}C at 10^{o}C/min and kept at this temperature for 15 min

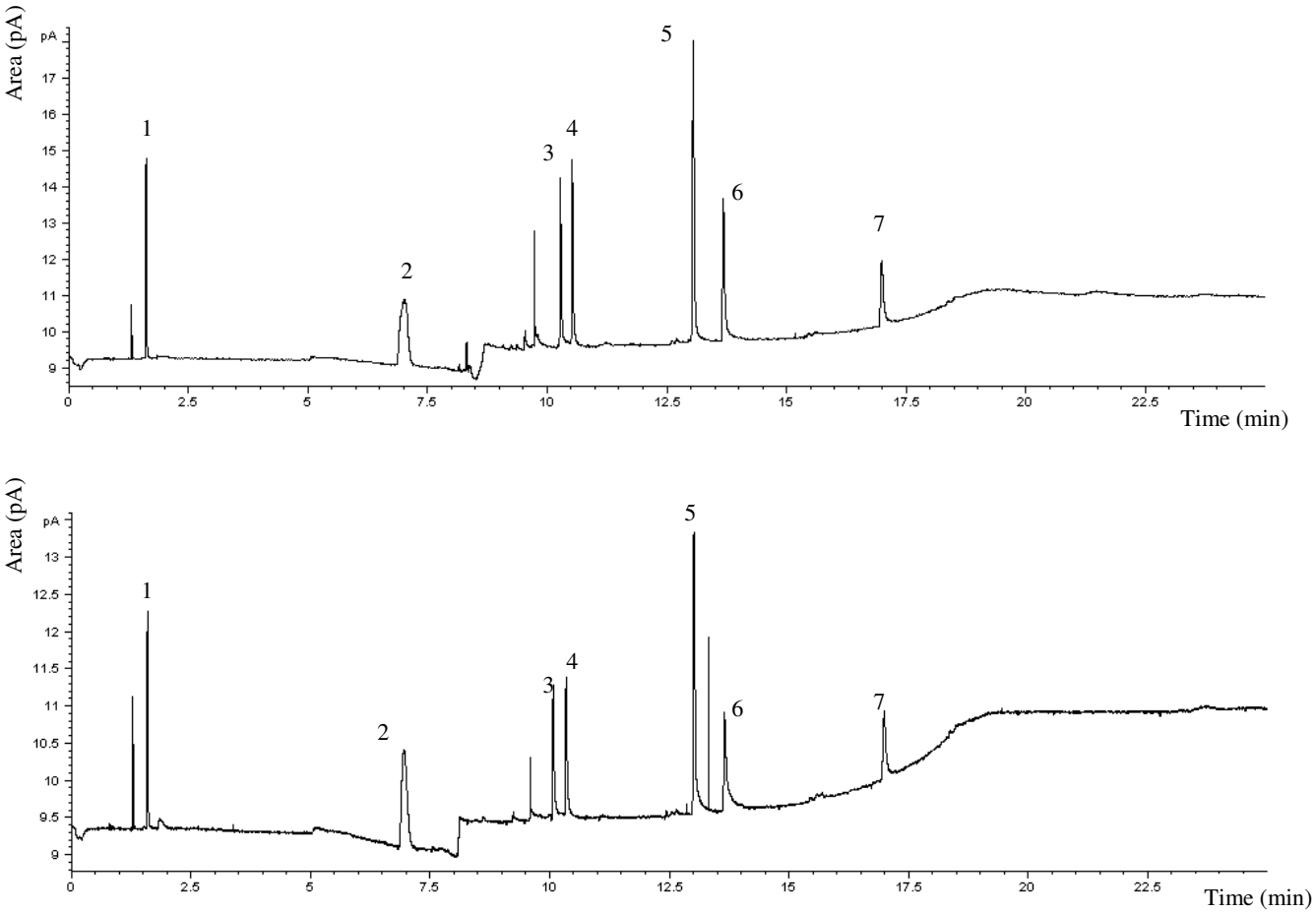

Figure 3. GC chromatogram of direct aqueous injection (DAI) water sample spiked with 1000 mg/m^3 volatile fatty acids (VFAs) and 4-methyl phenol, 498 mg/m^3 3-methyl indole, 1500 mg/m^3 1–butanol and phenol and 1507 mg/m^3 dimethyl sulphide (DMS), using the temperature program for the 1-butanol: 35^{o}C for 5 min, then increased to 120^{o}C at 30^{o}C/min, then increased to 225^{o}C at 10^{o}C/min and kept at this temperature for 10 min. Two injection volume were used, top: 1.0 µl and bottom: 0.5 µl (1: DMS, 2: 1-butanol, 3: n-butyric acid, 4: iso-valeric acid, 5: phenol, 6: 4-methyl phenol, 7: 3-methyl indole)

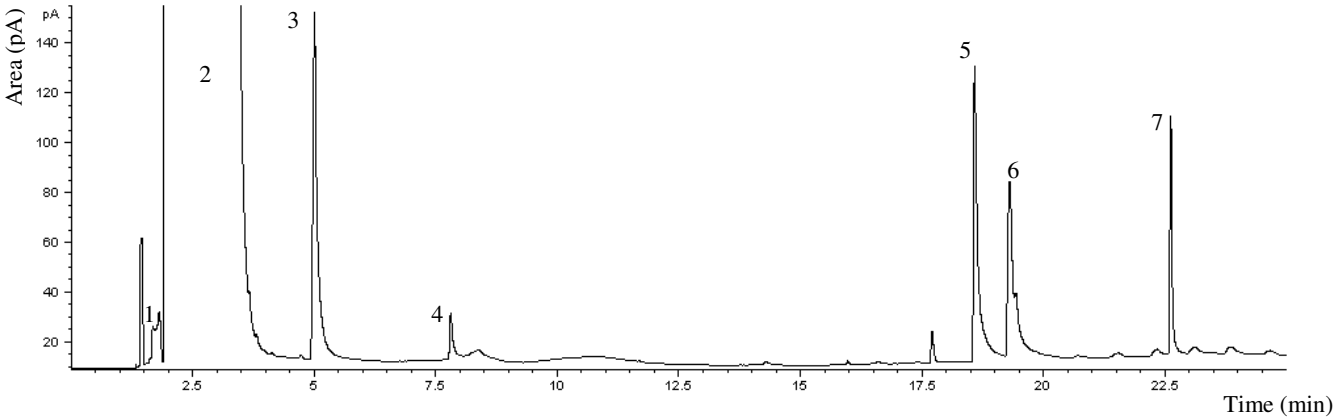

Figure 4. GC chromatogram of water sample extracted by Strata-X column. Water samples were spiked with 3750 mg/m^3 volatile fatty acids (VFAs) and 4-methyl phenol, 1867 mg/m^3 3-methyl indole, 5625 mg/m^3 1–butanol and phenol and 5653 mg/m^3 dimethyl sulphide (DMS) (1: DMS, 2: methylene chloride (solvent), 3: acetone (solvent), 4: 1-butanol, 5: phenol, 6: 4-methyl phenol, 7: 3-methyl indole). Injection volume: 1.0 µl. Temperature program: 35^{o}C for 5 min, then increased to 225^{o}C at 10^{o}C/min and kept at this temperature for 15 min

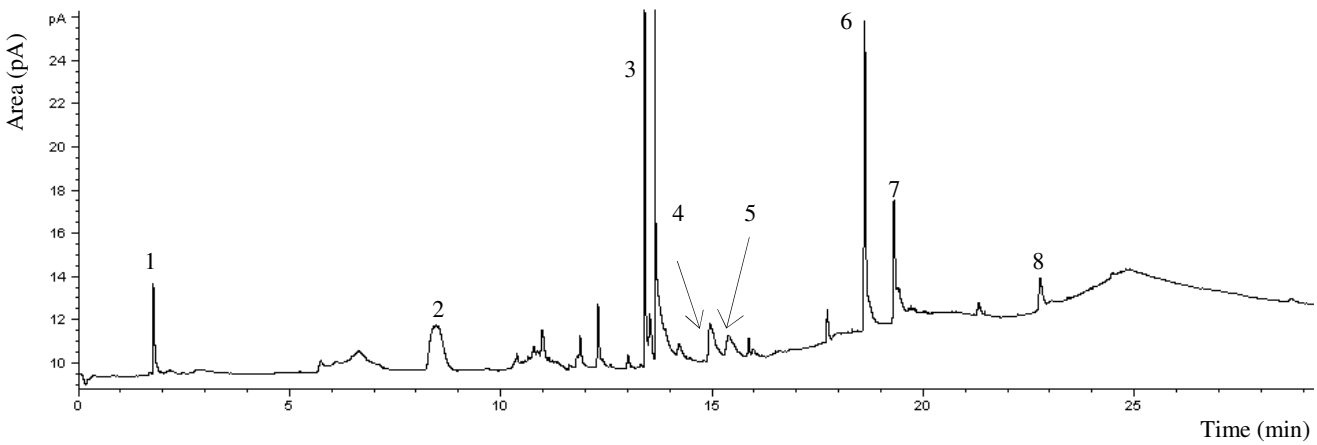

Figure 5. GC chromatogram of direct aqueous injection (DAI) for water sample from air wet scrubber in real Danish farm, spiked with target compounds (1: dimethyl sulphide (DMS), 2: 1-butanol, 3: formic acid, 4: n-butyric acid, 5: iso-valeric acid, 6: phenol, 7: 4-methyl phenol, 8: 3-methyl indole). Injection volume: 1.0 µl. Temperature program: 35^{o}C for 5 min, then increased to 225^{o}C at 10^{o}C/min and kept at this temperature for 15 min

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Paper II

Nawaf Abu-Khalaf, Jens Jørgen Lønsmann Iversen

Calibration of a sensor array (an electronic tongue) for identification and quantification of odorants from livestock buildings

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Calibration of a sensor array (an electronic tongue) for identification and quantification of odorants from livestock buildings

Nawaf Abu-Khalaf , Jens Jørgen Lønsmann Iversen*

Department of Biochemistry and Molecular Biology, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark

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)

∗ Corresponding author phone: +45 6550 2376; fax: +45 6550 2467, e-mail: jjli@bmb.sdu.dk

INTRODUCTION

An odour is defined as a sensation resulting from reception of a stimulus by the olfactory sensory system (Schiffman et al. 2001), and it is an important environmental pollution issue (Powers 2003). Odorants are the compounds responsible for imparting an odour, and their molecular mass is between 30 to 300 Daltons (Persaud et al. 1996; Sarig 2000). 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 (Schiffman et al. 1995).

There are many methods to reduce odours emission from livestock building, i.e. physical, chemical and biological. Biological methods are considered environmentally friendly (Revah and Morgan-Sagastume 2005). One of the biological methods is the bioscrubber which is used for air treatment in different industrial and agricultural applications (Hansen and Rindel 2000; Kraakman 2005). 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 (Revah and Morgan-Sagastume 2005).

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 (Abu-Khalaf et al. 2006; Kim et al. 2002; Razote et al. 2004; Shin and Ahn 2004). Analytical methods have the advantages of objectivity, repeatability and accuracy (Gostelow et al. 2001).

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 (Legin et al. 1997; Vlasov et al. 2002). It was already used in many applications including characterization of different types of mineral water and wine (Legin et al. 1999), monitoring of fermentation process (Legin et al. 2004; Turner et al. 2003) and food quality (Auger et al. 2005; Rudnitskaya et al. 2002).

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 (Gouma et al. 2004; Vlasov et al. 2002; Winquist et al. 2004). However, ET is a recently developed method, and it has not yet reached its full potential for application outside the laboratory (Soderstrom et al. 2003; Vlasov et al. 2002). Nevertheless, ET's ability should neither be underestimated (Vlasov et al. 2002) nor overestimated (Winquist et al. 2004), and more research should address new applications.

There are many advantages in using ET compared to other methods, such as GC, 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) (Legin et al. 2004; Soderstrom et al. 2003; Winquist et al. 2004).

There are huge numbers of odorants in the livestock building. Approximately 300 different odorants have been identified (Schiffman et al. 2001), many of them have a very low detection threshold of 0.001 mg/m^3 or less (O'Neil and Philips 1992). 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 (Singh and Ward 2005). However, most microbial growth occurs near neutral pH (McNevin and Barford 2000). 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.

EXPERIMENTAL

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 (Legin et al. 1999; Pravdova et al. 2002).

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 representive 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 (Kirk and Othmer 1991). 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 (Le et al. 2005).

Many researchers investigated the concentrations of these key odorants in air samples from livestock buildings. O'Neil and Philips (1992) and Schiffman et al. (2001) reviewed concen-

tration 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 (Datta and Allen 2005). 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 (Squillace et al. 1997). 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 (Budavari et al. 1996). All key odorants were used without any further purification.

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 (2001) 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.

Multivariate data analysis

Multivariate data analysis, including pattern recognition and calibration methods, is reviewed in many papers (Burns and Whitesides 1993; Despagne and Massart 1998; Jurs et al. 2000; Pravdova et al. 2002; Richards et al. 2002; Svozil et al. 1997).

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) (Jurs et al. 2000; Pravdova et al. 2002). 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 relation between samples) and loadings (which describes the relation between 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 (Legin et al. 1999; Pravdova et al. 2002).

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 (Pravdova et al. 2002; Wold et al. 2001). 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 (Dieterle et al. 2004).

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 (Lammertyn et al. 2000). 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 averaging triplicates of each sample.

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 (Burns and Whitesides 1993; Svozil et al. 1997). ANNs have many applications, for instance in spectroscopy, process control, protein folding, analytical chemistry and electrochemical systems (Richards et al. 2002; Svozil et al. 1997).

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 (Burns and Whitesides 1993; Despagne and Massart 1998). Principal components are used as inputs for the neural network model in order to reduce the risk of overfitting (Dieterle et al. 2004).

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 (Copper 2004). The models in the program contain one hidden layer with different numbers of nodes. Despange and Massart (1998) 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 (Copper 2004; Despagne and Massart 1998). 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 (1999) 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 (NeuralWare 2003).

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 (Despagne and Massart 1998) 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 before modelling in both PLS and BPNN, so each variable will have the same importance in the analysis (Wold et al. 2001). 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 Unscrambler and 'Pre-

dict' programs. 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. (1999) and Auger et al. (2005). 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 (Fearn 2002; Williams 2001).

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 (O'Neil and Philips 1992; Schiffman et al. 2001). 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 (Le et al. 2005).

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 (Arogo et al. 2003; Le et al. 2005). 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.

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) × 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 (Figure 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 elec-

trodes 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 (16 samples). The PCA score plot of the remaining samples, 34 samples, is shown in Figure 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.

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 (Figure 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 (Figure 4). It is noticed that slope, correlation, RMSEP and RPD showed an improvement in the BPNN model compared to the PLS-1 model.

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 (Figure 5).

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 (Figure 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 (Figure 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.

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 (Figure 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.

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 (Figure 8).

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 (Figure 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 (Figure 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 (Figure 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.

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 (Figure 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.

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 (Figure 13).

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) (Vlasov et al. 2005). The non-linear response of the electrodes results from interferences between ions in the test mixtures (Legin et al. 2004). 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. As expected the decreased, but sufficient number of electrodes improved the reproducibility, since the standard deviation and the RSD decreased, as shown in Table 5.

ET measured mainly ions in the mixtures (Soderstrom et al. 2005). 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 (Singh et al. 2005), or removal of one single compound only (Sheridan et al. 2003).

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 (Arogo et al. 2003; Timmer et al. 2005).

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 (Le et al. 2005).

By comparing the results of quantification using ET and gas chromatography (GC) (Abu-Khalaf et al. 2006), it is seen that ET could model phenolate from10^{-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.

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.

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 perform-

ance 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.

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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_2O 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 coeffi-cient (log p)	Melting point °C	Boiling point °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 (2005)

[a] *pKa* for indole (Kirk and Othmer 1991)

Table 2: Concentration of key odorants in air and water

Odorant	Dimensionless air-water partition coefficient	Minimum key odorant concentration in air [c]	Maximum key odorant concentration in air [c]	Minimum equivalent equilibrium key odorant concentration in water [d, e]		Maximum equivalent equilibrium key odorant concentration in water [d, e]		Interval of concentrations used in experiments	
	$(K_{AW})^b$	mg/m^3	mg/m^3	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 (1992) and Schiffman et al. (2001)

[d] Equivalent equilibrium concentrations in water calculated using K_{AW} (Datta and Allen 2005):

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}	5×10^{-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

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 (Figure 1)
		2, 5, 6, 7, 8, 9	ammonium	I. between 10^{-7} - 10^{-3} M, when concentration of n-butyrate was < 10^{-4} M (Figure 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 (Figure 3 and Figure 4)
		2, 5, 6, 7, 8, 9	n-butyrate	Q. between 10^{-5} - 10^{-3} M, when concentration of ammonium was < 5×10^{-4} M (Figure 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 < 5×10^{-4} M (Figure 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 < 5×10^{-4} M (Figure 6 b)
6	Containing p-cresolate	1, 2, 4, 5, 8	n-butyrate	I. between 5×10^{-4} - 10^{-3} M (Figure 7)
		1, 2, 4, 5, 8	n-butyrate	Q. between 10^{-5} - 10^{-3} M (Figure 8)
8	Containing ammonium	1, 2, 4, 5, 7, 8	n-butyrate	I. between 5×10^{-4} - 10^{-3} M (Figure 9)
		1, 2, 4, 5, 7, 8	n-butyrate	Q. between 10^{-5} - 10^{-3} M (Figure 10)
		1, 5, 7, 8	phenolate	Q. between 10^{-6} - 10^{-5} M, when concentration of n-butyrate and ammonium were < 5×10^{-4} M (Figure 11)
8	Containing p-cresolate	2, 5, 6, 7, 8, 9	n-butyrate	I. between 5×10^{-4} - 10^{-3} M (Figure 12)
		2, 5, 6, 7, 8, 9	n-butyrate	Q. between 5×10^{-5} - 10^{-3} M (Figure 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

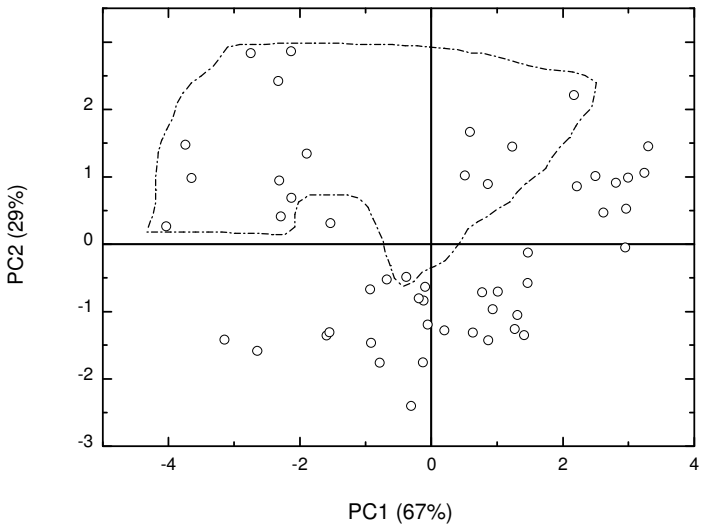

Figure 1. PCA score plot of all samples in test mixtures of key odorants containing ammonium at pH 6. Samples surrounded by dashed line (16 samples) contain high ammonium concentration (10^{-4} to 10^{-3} M). Full cross validation was used and six electrodes were sufficient

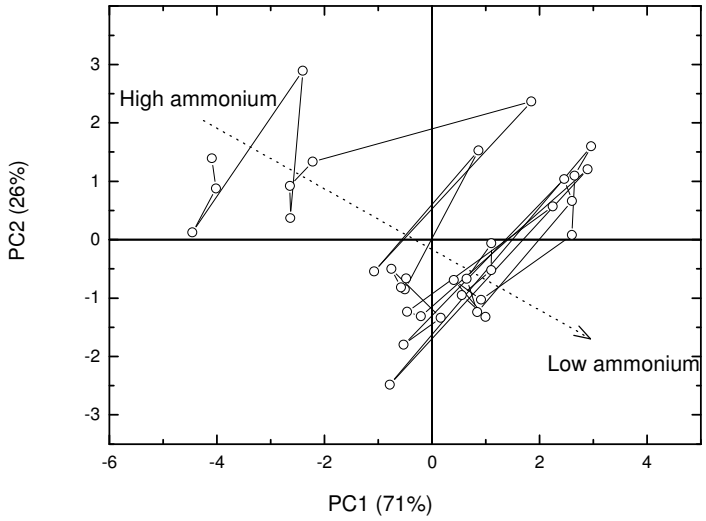

Figure 2. PCA score plot of 34 samples in test mixtures of key odorants containing ammonium at pH 6. Concentration of n-butyrate was below 10^{-4} M. Full cross validation was used and six electrodes were sufficient

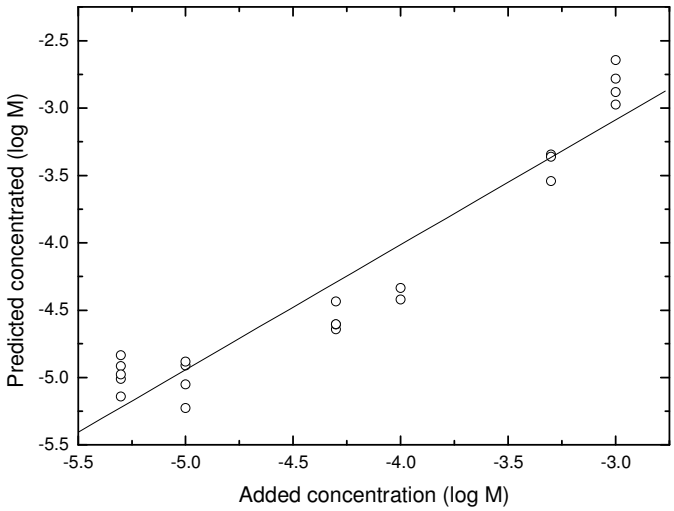

Figure 3. Calibration curve of ammonium from 5×10^{-6} to 10^{-3} M at pH 6. PLS-1, full cross validation for 22 samples and two PCs were used and six electrodes were sufficient. Concentration of n-butyrate was below 10^{-4} M

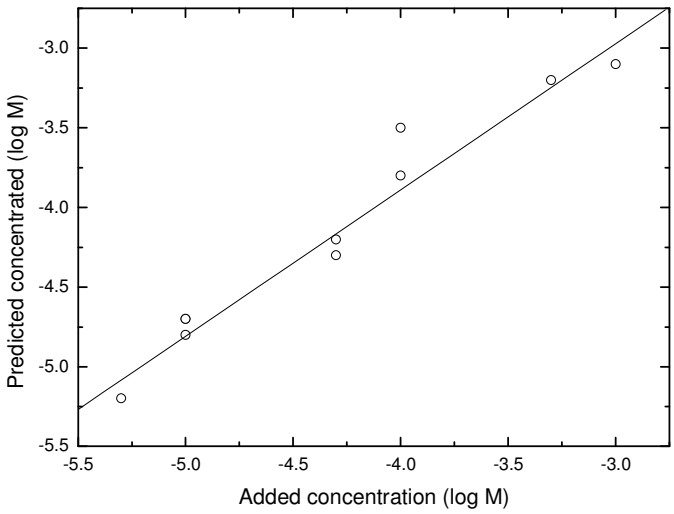

Figure 4. Calibration curve (18 samples) of ammonium from 5×10^{-6} to 10^{-3} M at pH 6. BPNN used 6, 3, 1 nodes. Concentration of n-butyrate was below 10^{-4} M

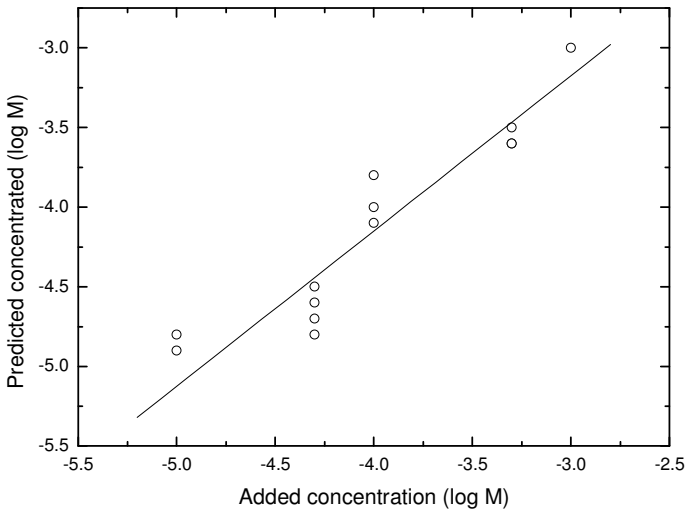

Figure 5. Calibration curve (18 samples) of n-butyrate at pH 6. BPNN used 6, 8, 1 nodes. Concentration of ammonium was below 5×10^{-4} M

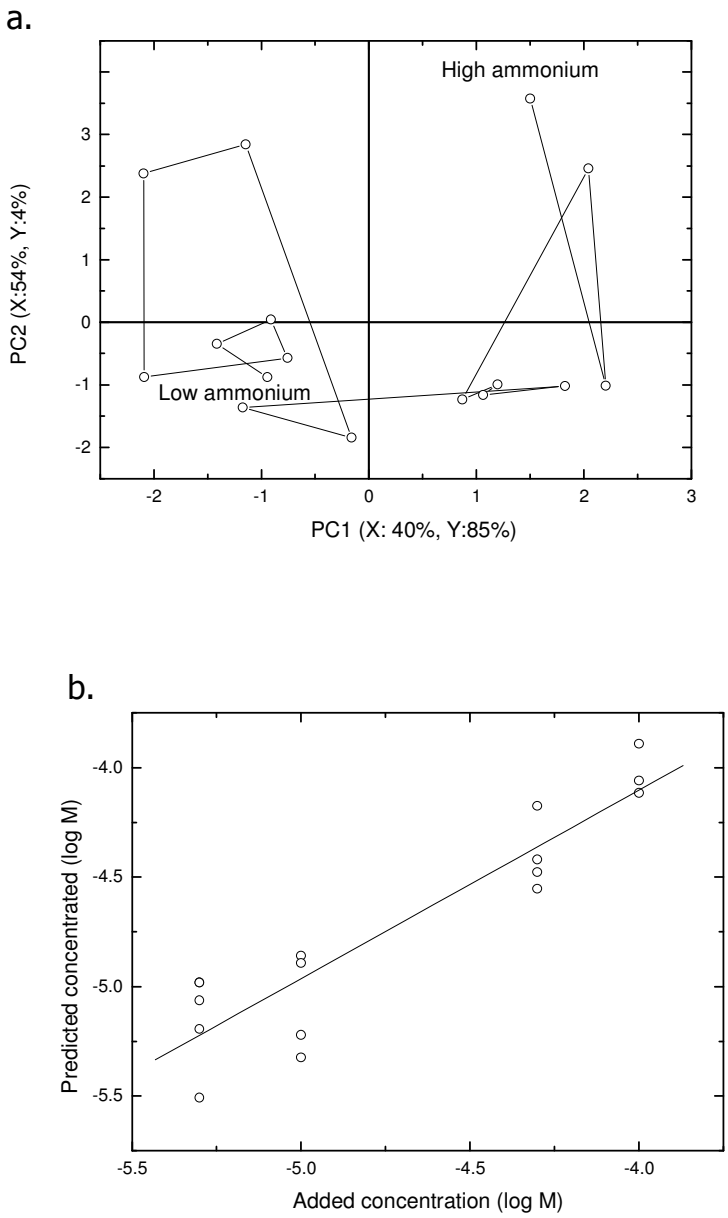

Figure 6 a: PLS-1 score plot for 16 samples of ammonium, considering limits of modelling in test mixtures of key odorants containing ammonium at pH 6. B: Calibration curve of identical ammonium samples. PLS-1, full cross validation and two PCs were used and six electrodes were sufficient

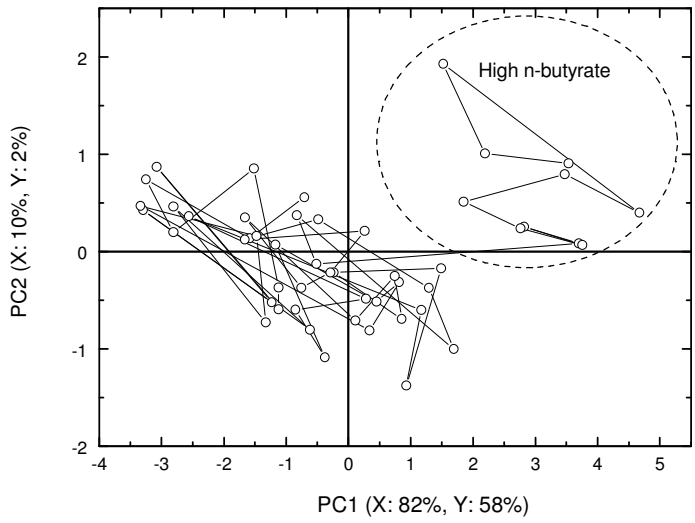

Figure 7. PLS-1 score plot of all samples in test mixtures of key odorants containing p-cresolate at pH 6. Samples (10 samples) with high concentrations (5×10^{-4} - 10^{-3} M) of n-butyrate are surrounded by dashed line. Full cross validation was used and five electrodes were sufficient

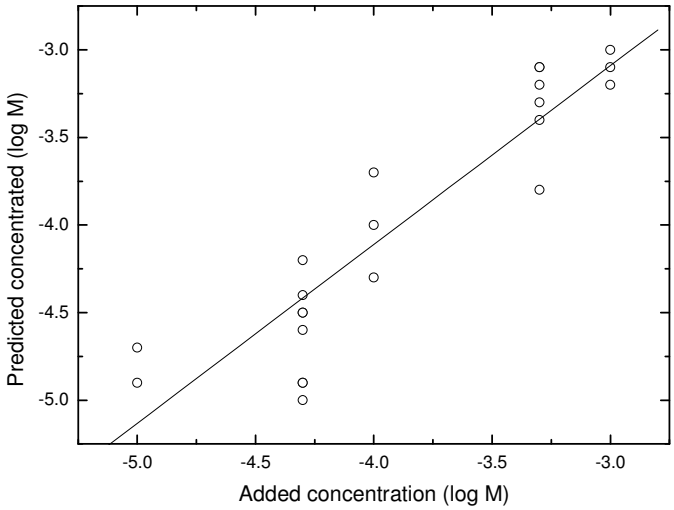

Figure 8. Calibration curve (27 samples) of n-butyrate in test mixtures of key odorants containing p-cresolate at pH 6. BPNN used 5, 2, 1 nodes

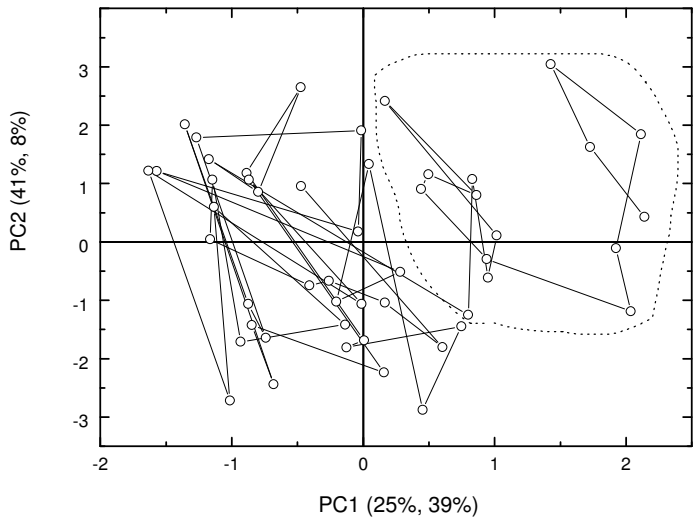

Figure 9. PLS-1 score plot of all samples in test mixtures of key odorants containing ammonium at pH 8. Samples (15 samples) with high concentrations (5×10^{-4} - 10^{-3} M) of n-butyrate are surrounded by dashed line. Full cross validation was used and six electrodes were sufficient

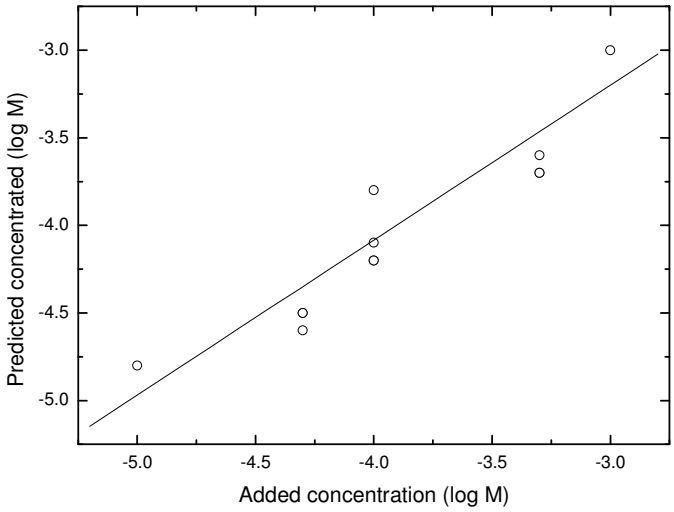

Figure 10. Calibration curve (21 samples) of n-butyrate in test mixtures of key odorants containing ammonium at pH 8. BPNN used 6, 0, 1 nodes

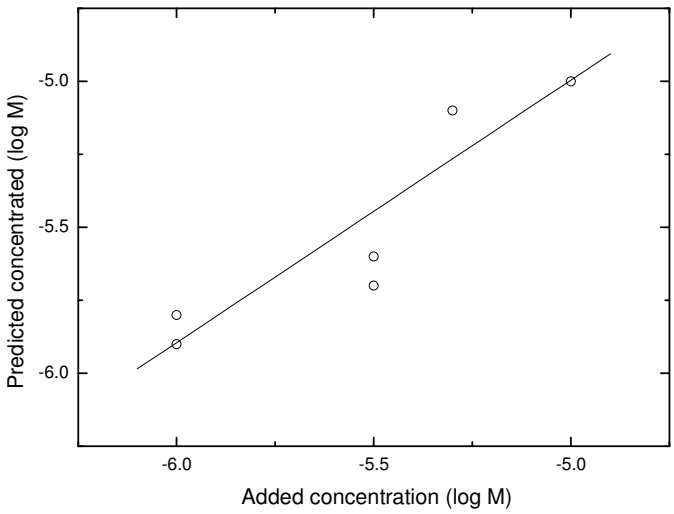

Figure 11. Calibration curve (12 samples) of phenolate in test mixtures of key odorants containing ammonium at pH 8. BPNN used 4, 4, 1 nodes

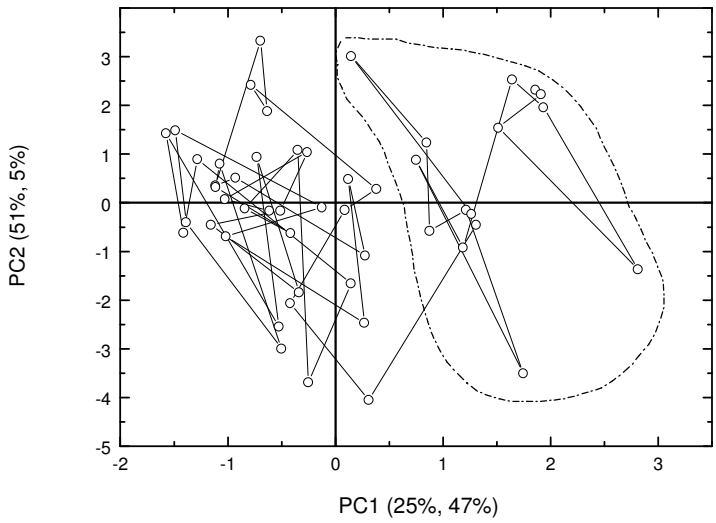

Figure 12. PLS-1 score plot of all samples in test mixtures of key odorants containing p-cresolate at pH 8. Samples (15 samples) with high concentrations (5×10^{-4} - 10^{-3} M) of n-butyrate are surrounded by dashed line. Full cross validation was used and six electrodes were sufficient

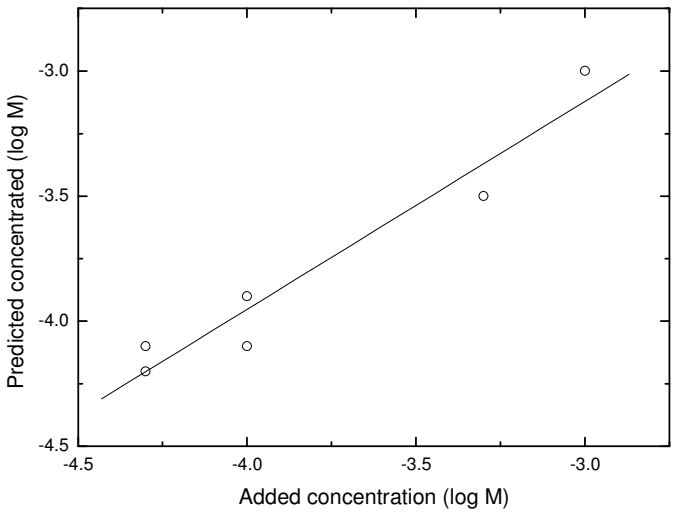

Figure 13. Calibration curve (18 samples) of n-butyrate in test mixtures of key odorants containing p-cresolate at pH 8. BPNN used 6, 9, 1 nodes

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Paper III

Nawaf Abu-Khalaf, Jens Jørgen Lønsmann Iversen

Classification of mixtures of odorants from livestock buildings by a sensor array (an electronic tongue)

Submitted to: Analytical Letters

Classification of mixtures of odorants from livestock buildings by a sensor array (an electronic tongue)

Nawaf Abu-Khalaf , Jens Jørgen Lønsmann Iversen*

Department of Biochemistry and Molecular Biology, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark

ABSTRACT

An electronic tongue comprising different numbers of electrodes was able to classify different test mixtures of key odorants (n-butyrate, iso-valerate, phenolate, p-cresolate, skatole and ammonium) with high performance in micromolar concentrations, which makes it suitable as an on-line sensor for characterization of odorants in livestock buildings.

Back propagation artificial neural network was used for classification. The average classification rate was above 80% in all cases.

A limited, but sufficient number of electrodes were selected by average classification rate and relative entropy. The sufficient number of electrodes decreased standard deviation and relative standard deviation compared to the full electrode array.

Keywords: electronic tongue, odorants, classification, back propagation artificial neural network (BPNN), average classification rate (ACR)

* Corresponding author phone: +45 6550 2376; fax: +45 6550 2467, e-mail: jjli@bmb.sdu.dk

INTRODUCTION

An odour is defined as a sensation resulting from the reception of a stimulus by the olfactory sensory system (Schiffman et al. 2001). The odour emission from livestock buildings in intensive farming is causing many environmental and health problems (Schiffman et al. 1995).

Biological methods, which are environmentally friendly, are the preferred techniques for reducing emission of odours from livestock buildings. The bioscrubber is one of the biological methods and comprises an absorption column, in which the polluted air stream from the livestock building is washed by water droplets, and a bioreactor, which cleans and recycles the washing water coming from the absorption column (Revah and Morgan-Sagastume 2005).

Characterization of odorants, in absorption column or in bioreactor, is necessary in the optimization of the bioscrubber. Also, there is a demand for an alarm system for monitoring odorant in livestock buildings (Arogo et al. 2003; Timmer et al. 2005). It was recently observed that an electronic tongue (ET) has a high potential as an on-line sensor for odorants (Abu-Khalaf and Iversen 2006). ET is an analytical instrument containing an array of electrodes, with partial specificity for different components in liquids in addition to an appropriate pattern recognition or multivariate calibration tool for identification and quantification of even complex liquid mixtures (Legin et al. 1997; Vlasov et al. 2002). Recently, ET was used to classify different types of wine and water (Legin et al. 1999) and four molds and one yeast (Soderstrom et al. 2005).

The pH is an important control variable in the bioscrubber for two reasons. pH affects the transfer of odorants from the gas to the liquid phase in the absorption column, and it also affects the microbes in the bioreactor. The optimum pH in the bioreactor is in the range of 4 to 8 (Singh and Ward 2005). However, most microbial growth occurs near neutral pH (McNevin and Barford 2000).

The objective of this communication is to use an ET to classify different test mixtures of key odorants. For a detailed account of the calibration of the ET we refer to a previous communication (Abu-Khalaf and Iversen 2006). In livestock buildings, there are huge numbers of odorants (Schiffman et al. 2001). A representative selection of these odorants, called key odorants, was used in this study. The key odorants were selected to represent a variety of chemical groups and were n-butyrate (n-butanoate), iso-valerate, phenolate, p-cresolate, skatole and ammonium. ET was used to classify four test mixtures of key odorants, i.e. two

test mixtures of key odorants at two different acidities (i.e. pH 6 and 8). Moreover, ET was used to classify six different test mixtures of key odorants that were prepared to give the maximum representation of a variety of chemical groups at pH 6.

EXPERIMENTAL

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

A custom made prototype ET was purchased from Analytical Systems, Ltd., St. Petersburg – Russia. It consists of 14 potentiometric electrodes. Eleven polymer (PVC) plasticized membrane electrodes (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 the classification. A pH glass electrode and a conventional Ag/AgCl reference electrode were included in the ET. Potentiometric measurements were performed using a high-input impedance multichannel voltmeter connected to a PC for data acquisition.

Preparation of test mixtures of key odorants

The concentrations of odorants in air samples from livestock buildings were investigated by many researchers. O'Neil and Philips (1992) and Schiffman et al. (2001) reviewed concentration intervals which are used as the main reference for the minimum and maximum concentrations of these odorants. Odorants are transferred to the liquid phase in the bioscrubber. The equivalent equilibrium concentrations of key odorants in water were calculated by using the dimensionless air-water partition coefficient *(K_{AW})* (Datta and Allen 2005), as shown in Table 1.

Stock solutions of different concentrations were prepared separately for each key odorant in the test mixtures. Iso-valeric acid, n-butyric acid and p-cresol all had purities of 99%. Skatole and phenol were obtained as solids, and had purities of 98% and 99.5%, respectively. All odorants were purchased from Sigma-Aldrich (Schnelldorf, Germany). Ammonium hydroxide (25%, v/v) was purchased from J. T. Baker (Deventer, Holland). All the odorants were diluted in deionised water, except skatole which was dissolved in hot deionised water (Budavari et al. 1996). The odorants were used without any further purification.

Experimental design

Five groups of experiments were carried out separately. Data from the first four groups of experiments were also used for calibration of the ET (Abu-Khalaf and Iversen 2006). The

first test mixture of key odorants contained: n-butyrate, iso-valerate, phenolate, skatole and ammonium. In the second test mixture, ammonium was replaced with p-cresolate. Ammonium and p-cresol were chosen because of their importance as part of the odour problems in livestock buildings (Arogo et al. 2003; Le et al. 2005). At pH 6, deionised water was solvent. At pH 8, a buffer of KH_2PO_4 (3.7×10^{-3} M) and Na_2HPO_4 (78×10^{-3} M) was solvent. Each group of experiments comprised 50 measurements in triplicates. The intervals of concentrations of each odorant were subdivided into seven intervals, to get as many combinations as possible in the test mixtures. The total number of measurements was 600. Details of test mixtures are shown in Table 2.

In the fifth experiment, test mixtures of key odorants were prepared to give maximum representation of a variety chemical groups, i.e. volatile fatty acids (VFAs) mixed with phenols, VFAs mixed with skatole, VFAs mixed with ammonium, etc. (Table 3). The test mixtures were diluted in deionised water after which the acidity was adjusted to pH 6 with NaOH or HCl. After this adjustment, the pH remained constant throughout the experiment. Each combination of the test mixtures was subjected to 15 measurements in triplicates, a total of 270 measurements (Table 3). The interval of concentrations was divided into five subsets, which were chosen from the seven intervals used in the previous four experiments.

In each group of experiments the test mixtures were measured in random order. Microsoft office Excel 2000 (Microsoft Corporation, USA) software was used to randomize the concentrations levels (seven levels in the first four groups of experiments and five levels in the fifth) in each group of experiments, using a randomization and uniform distribution function (Abu-Khalaf and Iversen 2006).

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 with deionised water several times between measurements, until they reached a steady potential. It was suggested that washing of electrodes is one of the solutions to avoid drift problems of electrodes in ET (Holmberg et al. 2004).

Artificial neural networks

The architecture of artificial neural networks (ANNs) is inspired by the structure of the brain. However, the architectures used in ANNs have lost their biological inspiration (Burns and Whitesides 1993; Svozil et al. 1997). There are many types of ANNs. One of the most widely used network is back propagation artificial neural network (BPNN), which is also called feed forward network. It comprises many processing elements, i.e. nodes, which are

arranged in layers: an input layer, an output layer, and one or more layers in between, called hidden layers.

A neural network software 'Predict' (v. 3.13, NeuralWare, Pittsburgh, USA), which uses BPNN and works in the framework of Microsoft Excel, was used in this study. The models in the program contain one hidden layer with different numbers of nodes, which results in a stable model (Despagne and Massart 1998). Models have direct connections between input and output nodes. This enables the program to evaluate the need for a hidden layer. Moreover, models employ an adaptive gradient learning rule. A weight decay method is employed to reduce overfitting. The use of the default parameters of 'Predict' software is recommended (Maier and Dandy 1999). The software employs hyperbolic tangent transfer function in the hidden layer. It employs sigmoid and softmax transfer functions in the output layer to address prediction and classification problems, respectively. The default parameters and mathematical explanation of the functions are beyond the scope of this communication but they are described elsewhere (NeuralWare 2003).

In the present study, classification (supervised networks) of test mixtures of key odorants was carried out. The input (independent variable) was the electrode signals, and the correlated output (dependent variable) was the class of test mixture. One column was utilized for classification during data arrangement in Excel worksheet. It contained the class of the test mixture, e.g. test mixtures of key odorants containing ammonium at pH 6 were called class 1, test mixtures of key odorants containing p-cresol at pH 6 were called class 2, etc. This method is called one-of-N encoding (NeuralWare 2003). 'Predict' software is able to distinguish between the text that presents classes in the classification problems, i.e. class 1, class 2, etc., and to identify the output nodes according to number of classes that were included in the classification model.

The classification rate for each test mixture of key odorants and the average classification rate (ACR) were found. The average classification rate is the average of classification rates of all classes. The values of the classification rate and the ACR are shown directly in the software, and there is no need for any calculations.

In each case of classification, the data were divided into train, test and validation sets. There is little agreement among researchers about the number of samples in training set for BPNN analysis. Basheer and Hajmeer (2000) concluded that there are no mathematical rules for solving this problem. However, Daspagne and Massart (1998) suggested that the number of samples in the training set should be at least twice the total number of weights in the BPNN topography. The latter recommendation was followed in this study.

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 before classification, so each variable contributes equally in the analysis (Wold et al. 2001).

A higher ACR and a lower relative entropy are the most important factors for classification problems using 'Predict' software (Copper 2004). The relative entropy is an internal measurement in the 'Predict' classification model. It measures the shared information between probability distributions. The higher this value is, the more similar the probability distributions are.

All electrodes were examined for their individual contribution to classification of test mixtures of key odorants. The goal was to achieve the highest ACR and the lowest relative entropy with the minimum number of electrodes for further classification processes. Initially all (14) electrodes were investigated for classification, and ACR and relative entropy were determined. By statistical analysis of the outputs of many combinations of a decreased number of electrodes it was observed that eight electrodes were sufficient for classifying all test mixtures of key odorants without influencing negatively ACR and relative entropy. The total number of electrodes in the ET was reduced without any loss of analytical information. This was done before in many applications of ET, e.g. Auger et al. (2005) and Soderstrom et al. (2005).

RESULTS AND DISCUSSION

Classification of test mixtures of key odorants at pH 6

Standard deviation of triplicate measurements in the test mixtures of key odorants containing ammonium was between 0 - 11 mV and 0 - 6.6 mV when electrodes no. 1-14 and no. 1, 2, 5, 6, 7, 8, 9, 11 were used, respectively. The relative standard deviation (RSD = (standard deviation / mean) × 100), was between 0 - 4.8% and 0 - 3.4% when electrodes no. 1-14 and no. 1, 2, 5, 6, 7, 8, 9, 11 were used, respectively. For the test mixtures of key odorants containing p-cresolate, standard deviation was between 0 - 17.3 mV and 0.1 - 6.8 mV when electrodes no. 1-14 and no. 1, 2, 5, 6, 7, 8, 9, 11 were used, respectively. The RSD was between 0 - 15.5% and 0 - 3.5% when electrodes no. 1-14 and no. 1, 2, 5, 6, 7, 8, 9, 11 were used, respectively.

The data of each test mixture of key odorants were split into train, test and validation sets. The number of different samples was 30, 10 and 10 (i.e. 90, 30 and 30 including triplicates), respectively for each test mixture of key odorants. The BPNN used 8, 4, 2. Electrodes no. 1,

2, 5, 6, 7, 8, 9, 11 were sufficient. The classification rate for the validation set of the test mixtures of key odorants containing ammonium and test mixtures of key odorants containing p-cresolate was 80% and 97%, respectively. The ACR was 88%.

Classification of test mixtures of key odorants at pH 8

Standard deviation of triplicate measurements in the test mixtures of key odorants containing ammonium was between 0 - 2.6 mV and 0 - 1.6 mV when electrodes no. 1-14 and no. 1, 2, 5, 6, 7, 8, 9, 11 were used, respectively. The RSD was between 0 - 8.4% and 0 - 0.7% when electrodes no. 1-14 and no. 1, 2, 5, 6, 7, 8, 9, 11 were used, respectively. For the test mixtures of key odorants containing p-cresolate, standard deviation was between 0 - 2.1 mV and 0 - 1.6 mV when electrodes no. 1-14 and no. 1, 2, 5, 6, 7, 8, 9, 11 were used, respectively. The RSD was between 0 - 0.4% when electrodes no. 1, 2, 5, 6, 7, 8, 9, 11 were used. 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 the glass electrodes were omitted from the array, the RSD was between 0 - 0.9%.

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 deionised water at pH 6, i.e. reproducibility is higher. This is because the buffered mixture contains higher and stabilized concentrations of ions.

The data of each test mixture of key odorants were split into train, test and validation sets. The number of different samples was 30, 10 and 10 (i.e. 90, 30 and 30 including triplicates), respectively for each test mixture of key odorants. The BPNN used 8, 0, 2. Electrodes no. 1, 2, 5, 6, 7, 8, 9, 11 were sufficient. The classification rate for the validation set of both test mixtures was 100%, and consequently the ACR was 100%.

Classification of test mixtures of key odorants containing ammonium at pH 6 and pH 8

The data were split into train, test and validation sets as in the previous experiment. The BPNN used 8, 0, 2 nodes. Electrodes no. 1, 2, 5, 6, 7, 8, 9, 11 were sufficient. The classification rate for the validation set of both test mixtures was 100%, and consequently the ACR was 100%.

Classification of test mixtures of key odorants containing p-cresol at pH 6 and pH 8

The data were split into train, test and validation sets as in the previous experiment. The BPNN used 8, 0, 2 nodes. Electrodes no. 1, 2, 5, 6, 7, 8, 9, 11 were sufficient. The classification rate for the validation set of both test mixtures was 100%, and consequently the ACR was 100%.

Table 4 shows the classification rates and ACR for the validation sets of the different test mixtures of key odorants. ET signals respond mainly to ions in the test mixtures (Soderstrom et al. 2005). 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. 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 results in Table 4 indicate that ET has a promising potential as a sensor for odorants. ET signals contained the fingerprints for each test mixtures of key odorants, which explains the successful classification.

Classification of test mixtures of key odorants comprising maximum number of combinations of a variety of chemical groups at pH 6

Standard deviation of triplicate measurements in the test mixtures of key odorants shown in Table 3 was between 0 - 3.3 mV and 0.1 - 3.0 mV when electrodes no. 1-14 and no. 1, 2, 5, 6, 7, 8, 9, 11 were used, respectively. The RSD was between 0 - 2.3% and 0 - 1.2% when electrodes no. 1-14 and no. 1, 2, 5, 6, 7, 8, 9, 11 were used, respectively. Standard deviation is lower, i.e. reproducibility is better in comparison to the previous four experiments that were carried out in deionised water. This is because the complexity of the test mixtures, i.e. the number of key odorants, was reduced in the test mixtures of key odorants in this experiment.

The total number of samples (comprising triplicates) was 90, which is equivalent to 270 measurements, i.e. 6 test mixtures $\times$ 15 samples $\times$ 3 (triplicates). The data were split into train, test and validation sets. The number of different samples was 42, 18 and 30 (i.e. 126, 54 and 90 including triplicates), respectively. Train, test and validation samples within each class of test mixtures of key odorants were considered. The number of different samples was 7, 3 and 5 (i.e. 21, 9 and 15 including triplicates), respectively. BPNN used 8, 4, 6 nodes. Electrodes no. 1, 2, 5, 6, 7, 8, 9, 11 were sufficient. The classification rates are shown in

Figure 1.Two test mixtures of key odorants having classification rate of 100%, contained VFAs and phenols, or phenols and ammonium, i.e. A and F, respectively. The test mixtures of key odorants that contained VFAs and ammonium, i.e. C, had the lowest classification rate (67%). The ACR for all test mixtures of key odorants was 81%. Most of the test mixtures of key odorants were misclassified as test mixtures C. However the objective of BPNN classification was to get the highest classification rate with lowest entropy. In the case of misclassifications, the test mixtures of key odorants were misclassified to only one different test mixture of key odorants, e.g. C was misclassified as F, and D was misclassified as E. This indicates that the classification model enables us to predict the class of the test mixtures of key odorants with an acceptable inaccuracy, e.g. C is only classified as C or F, and D is only classified as D or E.

When we tested numbers of electrodes that were less than the sufficient 8 electrodes used for classification, ACR decreased in comparison with the full array (14 electrodes) , e.g. when electrodes no. 2, 5, 6, 7, 8, 9 were used, the ACR decreased from 81% to 70%.

If pH changed when the test mixtures of key odorants were diluted in deionised water, adjustment of pH to 6 was carried out with NaOH or HCl. After adjustment, pH stayed constant throughout the measurement period. This is expected, since the VFAs in the test mixtures have buffer capacity.

BPNN classification models were superior to linear classification methods, e.g. Partial Least Square – Discriminant Analysis (PLS-DA) (Legin et al. 2004b).This was explained by the non-linear response of electrodes (Vlasov et al. 2005), which results from interferences between ions in the test mixtures (Legin et al. 2004a). However, PLS-DA showed a complete agreement with BPNN in some cases. PLS-DA was carried out for classification of the last three test mixtures of key odorants shown in Table 4. In these cases, the two test mixtures were easily separated in the PLS score plots, as shown in Figure 2 to Figure 4. Electrodes no. 1, 2, 5, 6, 7, 8, 9, 11 were sufficient.

Eight electrodes were sufficient for classification of all test mixtures of key odorants. Models using these eight electrodes resulted in the highest ACR and lowest entropy in comparison to any other number of electrodes. Also, standard deviation and RSD of triplicate measurements, i.e. reproducibility, improved when the number of electrodes was decreased (Table 5).

The sufficient number of electrodes for classification was determined from ACR and relative entropy in this work. For comparison, in calibration experiments (Abu-Khalaf and

Iversen 2006) the determination of the sufficient number of electrodes was based on many factors related to calibration curves, i.e. high correlation, reasonable slope, low root mean square error of prediction (RMSEP) and high ratio of standard error of performance to standard deviation (RPD). Therefore, the determination of sufficient number of electrodes in the classification process is much easier than in the calibration process.

Comparing the standard deviation and RSD of the sufficient number of electrodes used for calibration (Abu-Khalaf and Iversen 2006) and classification (this communication), it is obvious that the sufficient number of electrodes in the ET improved the reproducibility in comparison with the ET comprising 14 electrodes (Table 5).

In this and in an earlier communication (Abu-Khalaf and Iversen 2006) we have described the classification and calibration, respectively, of the ET. We have used test mixtures for this purpose, in order to simplify calibration and classification, respectively of up to five key odorants in a wide range of concentrations. Nine electrodes in total (no. 1, 2, 4, 5, 6, 7, 8, 9, 11) were sufficient for identification, quantification (Abu-Khalaf and Iversen 2006) and classification of all test mixtures of key odorants (this communication).

Next step of the application of the ET is to add these key odorant to liquid samples from the bioscrubber in order to measure recoveries. However, the liquid from the bioscrubber contains many and at present unknown compounds, which most likely will destroy the electrode membranes irreversibly. We want to avoid this fouling by separation of the bioscurbber liquid from the ET by introducing a membrane that will allow passage of key odorants only, but withhold fouling components. At present we are working on development of a membrane material for this purpose, a prerequisite for application of the ET as an on-line control sensor in bioscrubbers.

CONCLUSION

A calibrated ET, comprising 8 PVC plasticized cross-sensitive potentiometric electrodes, has successfully classified different test mixtures of key odorants. The ET was able to distinguish between two test mixtures of key odorants at the same pH with classification rates in the range of 88 - 100%. Classification between the same test mixtures of key odorants at different pH was even higher, 100%. Also, ET classified different test mixtures of key odorants comprising a variety of the chemical groups at pH 6. As expected the reproducibility of electrodes was better in this case, where the complexity of the mixture was decreased.

The results presented in this study are promising. The ability of ET to classify different test mixtures of key odorants with a high performance, makes ET an obvious candidate as an on-line sensor for characterization of odorants in livestock buildings.

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Table 1: Concentration of key odorants in air and water

Chemical group	Odorant	Henry's constant (H)	Dimensionless air-water partition coefficient	Minimum key odorant concentration in air [b]	Maximum key odorant concentration in air [b]	Minimum equivalent equilibrium key odorant concentration in water [c, d]		Maximum equivalent equilibrium key odorant concentration in water [c, d]	
		atm. l. mol^{-1}	(K_{AW}) [a]	mg/m^3	mg/m^3	mg/m^3	M	mg/m^3	M
VFA [e]	n-butyric acid	5.35×10^{-4}	2.19×10^{-5}	0.001	0.7	46	5.2×10^{-7}	32×10^{3}	3.6×10^{-4}
	iso-valeric acid	8.33×10^{-4}	3.40×10^{-5}	0.002	0.21	59	5.8×10^{-7}	62×10^{2}	6.0×10^{-5}
Phenol	phenol	3.33×10^{-4}	1.36×10^{-5}	0.001	0.0078	73	7.8×10^{-7}	57×10^{1}	6.1×10^{-6}
	p-cresol	1×10^{-3}	4.09×10^{-5}	0.002	0.041	49	4.5×10^{-7}	10×10^{2}	9.3×10^{-6}
Indole	skatole	2.13×10^{-3}	8.70×10^{-5}	0.00049	0.003	5.6	4.3×10^{-8}	34	2.6×10^{-7}
Ammonia	ammonia	1.61×10^{-2}	6.54×10^{-4}	0.01	18	15	8.9×10^{-7}	27×10^{3}	1.6×10^{-3}

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

$K_{AW} = H$ (atm. l. / mol) / 24.47

[b] According to O'Neil and Philip (1992) and Schiffman et al. (2001)

[c] Equivalent equilibrium concentrations in water calculated using K_{AW} (Datta and Allen 2005):

K_{AW} = Concentration in air (C_a) / Concentration in water (C_w) $\Rightarrow C_w = (24.47 \times C_a) / H$ (atm. l. /mol)

[d] M (mole/l.) = 10^{-6} × concentration (mg/m^3) / molecular mass (g/mole)

[e] VFA: volatile fatty acids

Table 2. Test mixtures of key odorants in four groups of experiments

Test mixtures of key odorants	Odorants present in test mixture	pH	Interval of concentrations (M)	Number of key odorants in test mixtures	Number of measurements
containing ammonium	n-butyrate	6	10^{-7} - 10^{-3}	5	150 (50 in triplicates)
	iso-valerate		10^{-7} - 10^{-4}		
	skatole		10^{-8} - 10^{-6}		
	phenolate		10^{-7} - 10^{-5}		
	ammonium		10^{-7} - 10^{-3}		
containing p-cresolate	n-butyrate	6	10^{-7} - 10^{-3}	5	150 (50 in triplicates)
	iso-valerate		10^{-7} - 10^{-4}		
	skatole		10^{-8} - 10^{-6}		
	phenolate		10^{-7} - 10^{-5}		
	p-cresolate		10^{-7} - 10^{-5}		
containing ammonium	same as above	8	same as above	5	150 (50 in triplicates)
containing p-cresolate	same as above	8	same as above	5	150 (50 in triplicates)

Table 3. Test mixtures of key odorants comprising a variety of chemical groups of selected key odorants at pH 6

Arbitrary name of test mixtures of key odorants	Groups of key odorants in test mixtures	pH	Key odorants in test mixtures	Interval of concentrations (M)	Numbers of key odorants in test mixtures	Number of measurements
A	VFAs + phenols	6	n-butyric acid	10^{-6} - 5×10^{-4}	4	45 (15 in triplicates)
			iso-valeric acid	5×10^{-7} - 5×10^{-5}		
			phenol	5×10^{-7} - 10^{-5}		
			p-cresol	5×10^{-7} - 10^{-5}		
B	VFAs + skatole	6	n-butyric acid	10^{-6} - 5×10^{-4}	3	45 (15 in triplicates)
			iso-valeric acid	5×10^{-7} - 5×10^{-5}		
			skatole	3×10^{-8} - 5×10^{-7}		
C	VFAs + ammonium	6	n-butyric acid	10^{-6} - 5×10^{-4}	3	45 (15 in triplicates)
			iso-valeric acid	5×10^{-7} - 5×10^{-5}		
			ammonium	10^{-6} - 5×10^{-4}		
D	phenols + skatole	6	phenol	5×10^{-7} - 10^{-5}	3	45 (15 in triplicates)
			p-cresol	5×10^{-7} - 10^{-5}		
			skatole	3×10^{-8} - 5×10^{-7}		
E	skatole + ammonium	6	skatole	3×10^{-8} - 5×10^{-7}	2	45 (15 in triplicates)
			ammonium	10^{-6} - 5×10^{-4}		
F	phenols + ammonium	6	phenol	5×10^{-7} - 10^{-5}	3	45 (15 in triplicates)
			p-cresol	5×10^{-7} - 10^{-5}		
			ammonium	10^{-6} - 5×10^{-4}		

Table 4. Classification rates and average classification rate (ACR) for validation sets of test mixtures of key odorants

Test mixtures of key odorants		Containing ammonium	Containing p-cresolate	Containing ammonium	Containing p-cresolate
	pH	6	6	8	8
Containing ammonium	6	80%	20%		
Containing p-cresolate	6	3%	97%		
ACR		88%			
Containing ammonium	8			100%	0%
Containing p-cresolate	8			0%	100%
ACR				100%	
Containing ammonium	6	100%		0%	
Containing ammonium	8	0%		100%	
ACR			100%		
Containing p-cresolate	6		100%		0%
Containing p-cresolate	8		0%		100%
ACR				100%	

Table 5. Standard deviation (StDev) and relative standard deviation (RSD) of triplicate measurements with different number of electrodes used for classification and calibration

pH	Test mixture of key odorants	Electrode no.	StDev [h] (mV)	RSD [i] (%)
6	Containing ammonium	1-14	0 - 11	0 - 4.8
		1, 2, 5, 6, 7, 8, 9, 11	0 - 6.6	0 - 3.4
		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, 5, 6, 7, 8, 9, 11	0.1 - 6.8	0 - 3.5
		1, 2, 4, 5, 8	0 - 6.8	0 - 3.5*
8	Containing ammonium	1-14	0 - 2.6	0 - 8.4
		1, 2, 5, 6, 7, 8, 9, 11	0 - 1.6	0 - 0.7
		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 [j]
		1-11, 14	0 - 2.1	0 - 0.9
		1, 2, 5, 6, 7, 8, 9, 11	0 - 1.6	0 - 0.4
		2, 5, 6, 7, 8, 9	0 - 1.6	0 - 0.4*
6	Test mixtures of key odorants comprising a variety of chemical groups at pH 6	1-14	0 - 3.3	0 - 2.3
		1, 2, 5, 6, 7, 8, 9, 11	0.1 - 3.0	0 - 1.2

[h] StDev: Standard deviation of triplicate measurements

[i] RSD: Relative standard deviation of triplicate measurements

[j] Potential readings and standard deviation were very small, which results in high value of RSD

* Data from Abu-Khalaf and Iversen (2006)

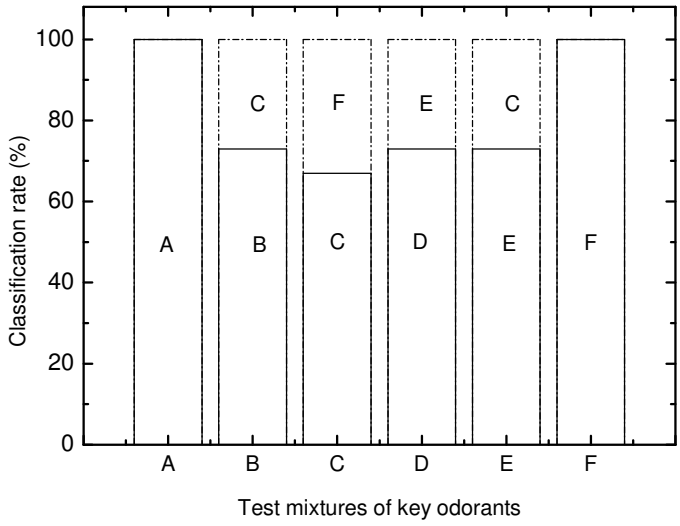

Figure 1. Classification rates for validation sets of different test mixtures of key odorants[f] comprising a variety of chemical groups at pH 6. Average classification rate (ACR) was 81%

[f] A: VFAs + phenols

B: VFAs + skatole

C: VFAs + ammonium

D: phenols + skatole

E: skatole + ammonium

F: phenols + ammonium

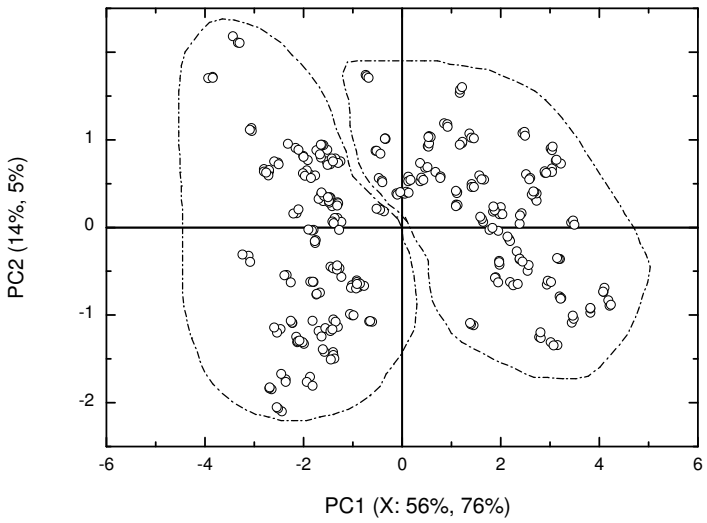

Figure 2. PLS-1 score plot of all samples in test mixtures of key odorants containing ammonium (to right) and test mixtures of key odorants containing p-cresolate (to left) at pH 8. Full cross validation, PLS-DA was used and eight electrodes were sufficient

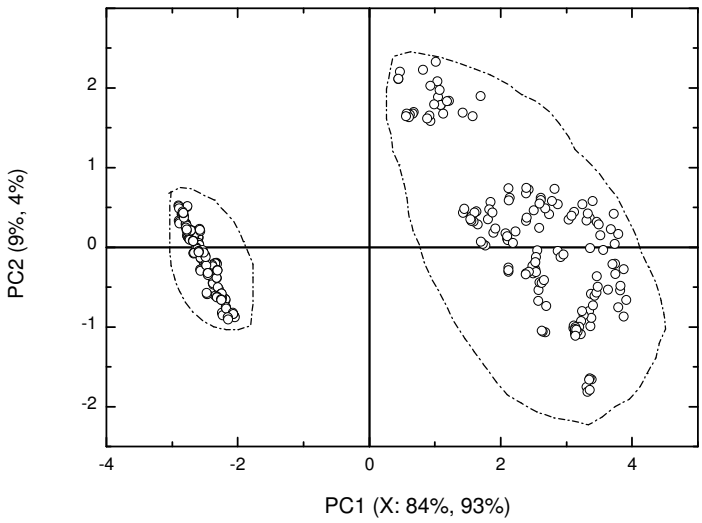

Figure 3. PLS-1 score plot of all samples in test mixtures of key odorants containing ammonium at pH 6 (to right) and at pH 8 (to left). Full cross validation, PLS-DA was used and eight electrodes were sufficient

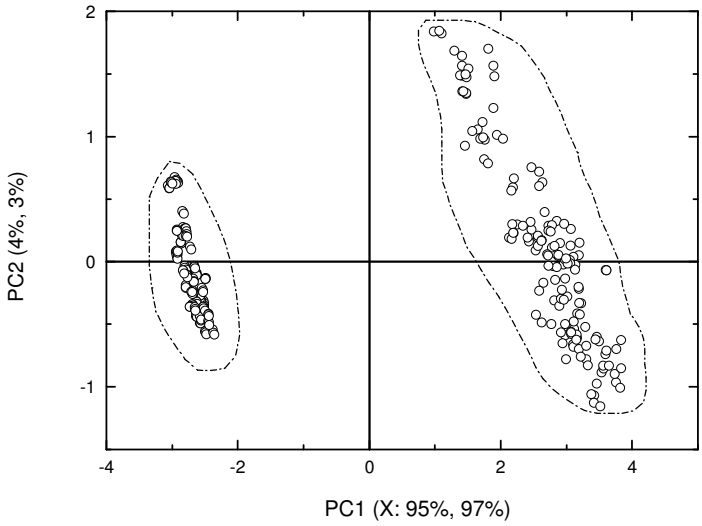

Figure 4. PLS-1 score plot of all samples in test mixtures of key odorants containing p-cresolate at pH 6 (to right) and at pH 8 (to left). Full cross validation, PLS-DA was used and eight electrodes were sufficient

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