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Potential for Detection of Microorganisms and Heavy Metals in Potable Water using Electronic Nose Technology

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Abstract

Studies have been carried out to determine the potential for the detection of different microbial species (*Enterobacter aerogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*), alone and in the presence of low concentrations of different heavy metals (As, Cd, Pb and Zn) in bottled, reverse osmosis (RO) and tap water, using an electronic nose. Studies show that it is possible to discriminate control water samples from water contaminated with 0.5 ppm of a mixture of metals. The presence of heavy metals may modify the activity of microorganisms and thus the volatile production patterns. Bacterial species at 10^2-10^4 colony forming units (CFUs) mL^{-1} could be detected after 24h of incubation. Work is in progress to identify the limits of detection for a range of other microorganisms, including, fungi and cyanobacteria, and chlorinated phenols using electronic nose technology.

Key words*: Gas sensor array; electronic nose; water contamination; microorganisms/heavy metals*.

1. Introduction

The rising costs of treating water and wastewater and the increasing requirement for clean water is prompting industry and authorities to examine new methods for water conservation. Conventional chemical methods used for the analysis of water and wastewater can be accurate, but are mainly based on sample collection and retrospective analyses. The recent availability of commercial sensor arrays (so-called electronic noses, e-nose) for detecting and characterizing headspace odours may offer a more rapid and relatively simple technique for monitoring changes in water and wastewater quality (Gostelow *et al*., 2001).

The e-nose mimics the human olfaction system which consists of three essential elements: an array of olfactory receptor cells situated in the roof of the nasal cavity, the olfactory bulb which is situated just above the nasal cavity, and the brain. In an e-nose there are three components; (a) a sampling conditioning unit, which delivers the odour volatiles from the head space of the sample, (b) a test chamber in which the sensor array is based; and (c) a processing unit which analyses the sensor responses for pattern recognition. The sensor array is composed of a certain number of sensors (6-32). The most common materials used for gas sensors are metal oxides, conducting polymers, and catalytic metals. The use of an array of non-specific sensors allows for the relative responses between the sensors to be used to produce a unique odour profile or fingerprint of an odour. The odour profile can then be further analysed using pattern recognition techniques and/or neural network algorithms (Stuetz *et al*., 1998).

The applications of e-nose technology have been widely reported for early detection and identification of microorganisms and signs of food spoilage (Pavlou *et al*., 2000; Magan *et al*., 2001; Keshri *et al*., 2002). The e-nose has also been used to detect the volatiles emitted by growing bacteria (Gibson *et al*., 1997; McEntegart *et al*., 2000). These studies have

generally been done with high concentrations of bacteria, centrifuged cell biomass, or colonies of bacteria grown on an agar surface. In view of these reports, the key issue with the use of the e-nose in microbial detection is sensitivity rather than selectivity.

Until now, the application of this technique in environmental measurements has been limited. For example, recently it was demonstrated that conducting polymer sensors can distinguish between distilled water and distilled water that contains low levels of organic pollutants, suggesting that such a system could be used to screen for tainting compounds in supply waters (Stuetz *et al*., 1998; Dewettinck *et al*., 2001; Ogawa and Sugimoto, 2002).

Currently pollution by heavy metals is one of our most serious environmental problems. Chemical contaminants in drinking water can be present together with numerous other inorganic and organic constituents. These can include cadmium, lead and zinc, as impurities from the household plumbing systems, in which the pipes, solder or fitting contain some of these metals (World Health Organization, 1996). So far, there are no studies using the e-nose technology to detect heavy metals in water.

The objectives of the present study was to investigate the potential use of an e-nose system employing a 14 conducting polymer sensor array, to sample the headspace volatiles originating from both microbial and metal aqueous solutions. The potential for differentiation between such contaminants was examined.

2. Experimental

2.1 Cultural media and incubation

The following bacterial species obtained from the UK National Culture Collections were used in this study: *Enterobacter aerogenes* (88), *Escherichia coli* (10000) and *Pseudomonas aeruginosa* (8672). A concentration of 10^2, 10^4 and 10^8 colony forming units (CFUs) mL $^{-1}$ for each species was incubated at 25ºC for a period of 24 h. These

concentration were obtained by correlating absorbance (640 nm, visible light) of different concentrations of cells and actual number of colonies using the spread plate technique.

For heavy metals, samples were prepared by adding a mixture of the heavy metals (As, Cd, Pb and Zn) to non-sterile water, to obtain a 10 ml aqueous mixed metal solution in two different concentrations, 0.5 and 2.0 ppm. Where the bacterial species were mixed with the heavy metals, the final concentrations were adjusted to 10^4 and 10^6 CFUs mL^{-1}. Samples were incubated for 24 hrs at 25^{o}C.

2.2 Gas sampling and sensing procedure

Each sample was connected with a specially made air-filter system, which consisted of a pair of 6 cm long Teflon tubing segments (Tygon), a bio-filter (0.45 μm, PTFE Whatman, HepaVent), and an activated carbon filter (Whatman) to ensure clean air flow above the aqueous solution samples headspace (Pavlou *et al*., 2000).

An electronic nose (model BH-114: Bloodhound Sensors Ltd., Leeds, UK), which employs 14 conducting polymer sensors, was used in this study. Activated carbon filtered air is passed over the sensor surface at a flow rate of 4 mL min^{-1} to generate the sensor baseline. The electronic nose was flushed and a one minute interval was allowed between each sample. Samples were analysed in a random pattern including the controls. Three replicates were used for each treatment and repeated twice.

2.3 Data analysis

The Bloodhound e-nose provides the following data for each of the 14 sensors. These are adsorption (maximum rate of change of resistance), desorption (maximum negative rate of change of resistance), divergence (maximum step response) and area (area under the actual sensor curve) (Pavlou *et al*., 2000). In this study the normalised data for divergence and area

were analysed using the program xlSTAT (Microsoft Excel add-in). Multivariate techniques such as Principal Components Analysis (PCA), Discriminant Function Analysis (DFA) and Cluster Analysis (CA) were applied to represent the sensors response.

3. Results and Discussion

Figure 1 shows the PCA obtained from normalised divergence and area data of the sensors response to two types of water, bottled and reverse osmosis (RO), treated with 0.5 ppm of a mixed metal solution (As, Cd, Pb and Zn), and RO water (non-sterile) was used as a control sample. It can be seen that the e-nose was able to distinguish tainted water samples from control ones. The non-sterile (control) water sample contains a low total population of bacteria (< 10-50 CFU ml^{-1}) and their activity would be modified by the presence of the heavy metals. This would account for modified volatile production patterns which were detected qualitatively by the e-nose.

Figure 2 represents the sensor responses to *E. coli* cells inoculated and un-inoculated with 0.5 ppm of the metal mixture mentioned above, and controls. There was a good separation between the controls (RO water), and samples containing bacteria plus metals and those containing bacteria only.

From the Discriminant Function Analysis (DFA) plots on the raw data values, it was possible to differentiate between control samples and those containing bacteria. Figure 3 shows the discrimination for *P. aeruginosa* incubated for 24 hours at 30ºC, and Figure 4 for *E. aerogenes* incubated for 48 hours at the same temperature. After 48 h a clear four-group separation was achieved. The inability to differentiate between 10^2 and 10^4 CFUs ml^{-1} treatments may be due to similar quantities of volatiles being produced in the water at these bacterial concentrations. This suggests that the limit of detection for these bacteria is between

10^2 and 10^4, and this group are clearly separated from the control. The results were consistent, as similar responses were obtained in repeat experiments on different days.

This is one of the first studies to examine the potential of using e-nose technology to differentiate water samples on the basis of qualitative volatile production pattern due to microbial or metal interaction. This study shows that potential does exists for detecting the presence of quite low concentrations (0.5 ppm) of important contaminants in water. The presence of low quantities of heavy metals may modify the activity of microorganisms and thus the volatile production patterns enabling further separation to be made. The use of non-sterile water can be the reason why it was possible to detect the presence of heavy metals in RO and bottled water. Fewtrell *et al*. (1997) carried out a survey on the microbiological quality of still bottled water. The results have revealed that almost 2% of the examined samples failed to meet the required microbiological standards.

The use of conducting polymer arrays for the discrimination of such samples has been successful in other media, e.g. milk-based liquid media where volatiles compounds produced by spoilage bacteria and yeasts using an initial inoculum of about $10^3 – 10^4$ CFU ml^{-1} (Magan *et al*., 2001) could be discriminated. McEntegart *et al*. (2000) reported the detection of bacteria in liquid media, of about $5x10^8$ cells ml^{-1}, using a mixture of three sensor types. So far, 10^2 - 10^4 CFUs ml^{-1} is the lowest level at which microbial contaminants can be detected using volatile production patterns. Other studies have tried to use the e-nose for real time monitoring of waste water streams by measuring changes in general odour production (Bourgeois *et al.*, 2001). Such approaches have been successful for the detection of fungal contaminants in grain where a prototype automated system was developed and found to operate effectively (Evans *et al*., 2000).

4. Conclusions

Potential exists for the early detection of microorganisms, and of low concentrations of heavy metals in different types of water. An opportunity also exists to extend this approach for the early detection of off-odours due to methylation of chlorinated phenols by fungi and actinomycetes which are important, especially in the summer months.

5. References

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Figure legends

Figure 1. PCA map of reverse osmosis (RO) and bottled water with 0.5 ppm of a mixed metal solution (As, Cd, Pb and Zn) discriminated from RO water (shaded area). Key: **C** - RO water; **R** - RO water with metals; **B** - bottled water with metals.

Figure 2. PCA of control samples and two cell concentrations of *E. coli* cells inoculated (shaded area) and un-inoculated with 0.5 ppm of a mixed metal solution (As, Cd, Pb and Zn), incubated for 24 h at 30ºC. Key: **C** - Control; **E4** - 10^4 CFU mL^{-1}; **E8** - 10^8; **M4** - 10^4 CFU mL^{-1} with metals; **M8** - 10^8 with metals.

Figure 3. DFA of *P. aeruginosa* and control samples, incubated for 24h at 30ºC. Key: **CO** - Control; **PT** - 10^2 CFU mL^{-1}; **PF** - 10^4; **PH** - 10^8.

Figure 4. DFA of *E. aerogenes* and control samples, incubated for 48h at 30ºC. Key: **CO** - Control; **AT** - 10^2 CFU mL^{-1}; **AF** - 10^4; **AH** - 10^8.

Figure 1

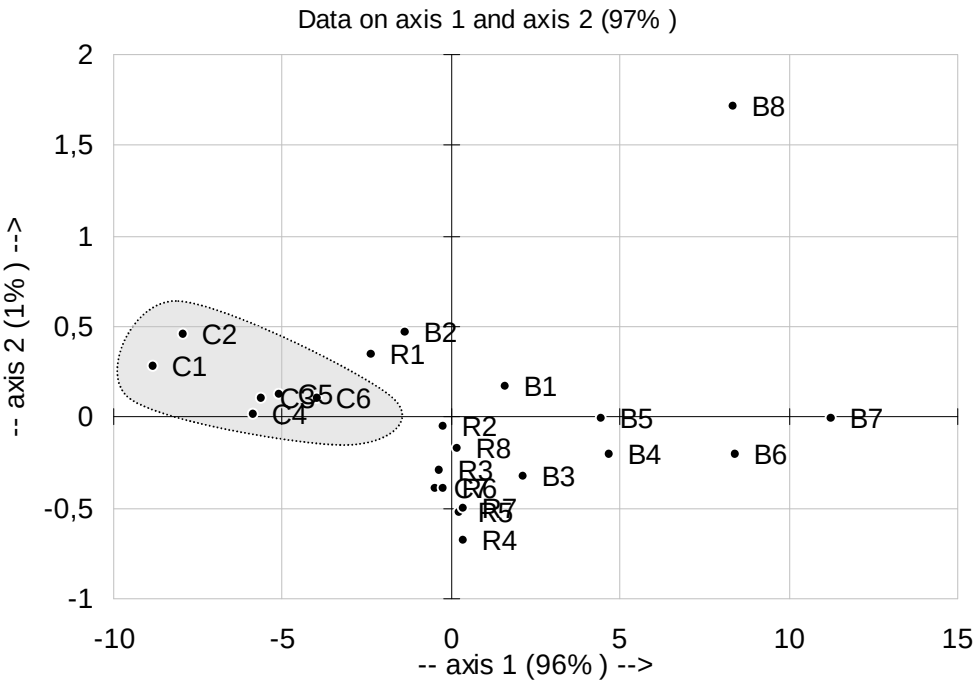

Data on axis 1 and axis 2 (97%)
2
1,5
1
0,5
0
-0,5
-1
-10
-5
0
5
10
15
-- axis 2 (1%) -->
-- axis 1 (96%) -->
B8
B2
R1
C2
C1
B1
C5
C6
C4
B5
B7
R2
R8
B4
B6
R3
B3
R4

Figure 2

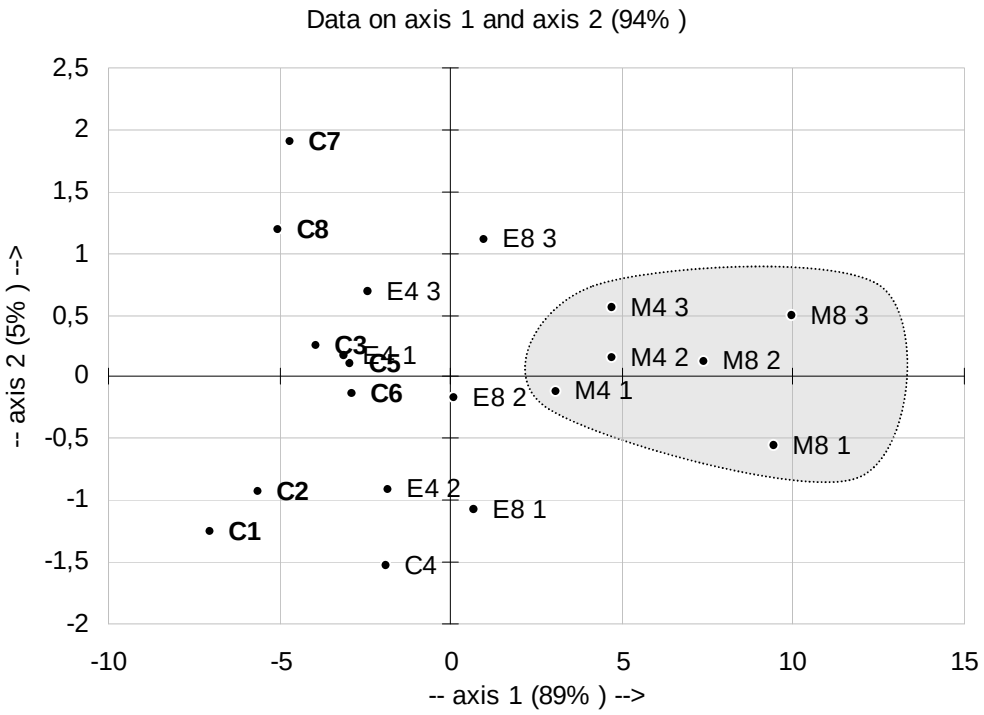

Data on axis 1 and axis 2 (94%)
2,5
2
1,5
1
0,5
0
-0,5
-1
-1,5
-2
-10
-5
0
5
10
15
-- axis 2 (5%) -->
-- axis 1 (89%) -->
C7
C8
E8 3
E4 3
M4 3
M8 3
C3
E4 1
C5
M4 2
M8 2
C6
E8 2
M4 1
M8 1
C2
E4 2
E8 1
C1
C4

Figure 3

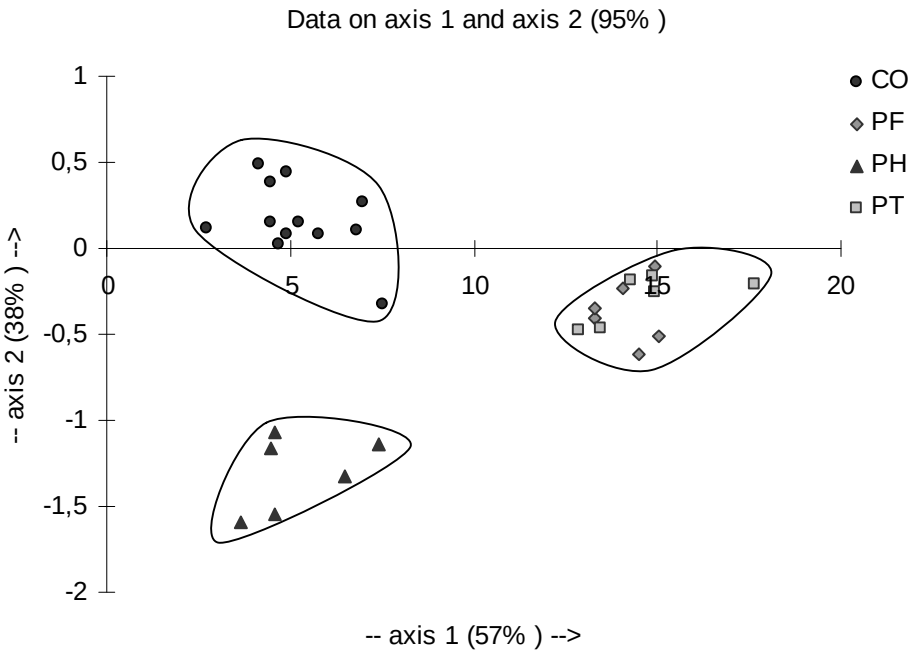
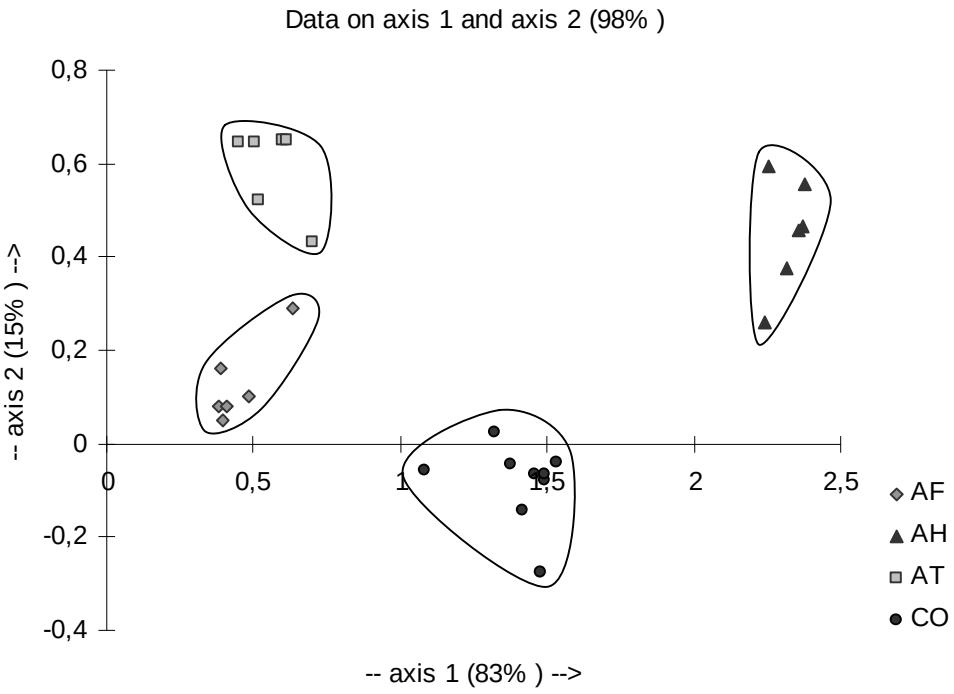

Data on axis 1 and axis 2 (95%)
CO
PF
PH
PT
1
0,5
0
-0,5
-1
-1,5
-2
0
5
10
15
20
-- axis 2 (38%) -->
-- axis 1 (57%) -->

Figure 4


Data on axis 1 and axis 2 (98%)
0,8
0,6
0,4
0,2
0
-0,2
-0,4
-- axis 2 (15%) -->
0
0,5
1
1,5
2
2,5
-- axis 1 (83%) -->
AF
AH
AT
CO

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