

*Review*

## **Application of Electronic Noses for Disease Diagnosis and Food Spoilage Detection**

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**Abstract:** Over the last twenty years, newly developed chemical sensor systems (so-called "electronic noses") have odour analyses made possible. This paper describes the applications of these systems for microbial detection in different fields such as medicine and the food industry, where fast detection methods are essential for appropriate management of health care. Several groups have employed different electronic noses for classification and quantification of bacteria and fungi to obtain accurate medical diagnosis and food quality control. So far, detection and identification of bacterial and fungal volatiles have been achieved by use of e-noses offering different correct classification percentages. The present review includes examples of bacterial and fungal species producing volatile compounds and correlated to infectious diseases or food deterioration. The results suggest the possibility of using this new technology both in medical diagnostics and in food control management.

**Keywords:** electronic noses, microorganisms, spoilage, clinical samples.

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### **1. Introduction**

The electronic nose instrumentation was developed in the early 1980s [1]. Since then, the analyses of volatile compounds have been of increasing interest and many studies have been dedicated to the improvement of odour measurements.

This technology aims to mimic the mammalian sense of smell by producing a composite response unique to each odorant. The system comprises a set of active materials that detects the odour and transduces the chemical vapours into electrical signals. The odour profile or "fingerprint" obtained can then be analysed using a pattern recognition program; statistical methods, e. g. Principal Components Analysis (PCA), Cluster Analysis (CA) and/or Neural Network Algorithms, are provided for data processing [2-3].

The first developed sensor array was a Metal Oxide Semiconductor (MOS), which detected 20 odours [1]. Technological advances made it possible to create sensor arrays (from six up to 32 sensors) with different materials, processing thousands of smells. This equipment comprises different types of sensors interacting with volatiles: Metal Oxide Semiconductor Field Effect Transistors (MOSFETs), Quartz Crystal Microbalances (QCMs), the Surface Acoustic Waves (SAWs), Conducting Organic Polymer Sensors (CPs), Intrinsically Conducting Polymers (ICPs) and optical fiber bundles [4-8]. Modern electronic noses (e-noses) can be constructed with more than one type of sensor in them. The development of gas chromatographic (GC) detectors together with direct column heating has recently produced a new type of ultra-fast gas chromatograph, called zNose<sup>TM</sup> [9-11]. All the devices listed above are designed for experimental procedures, data analysis and comparative evaluation of results (data treatment).

The applications of this technology range from environmental analysis and food processing to the pharmaceutical industry and medicine [12-16]. Electronic noses offer great potential for the detection of different microbial species. Some chemical products are specific to fungal and bacterial species and are commonly used as a useful diagnosis tool. Recently, it has been shown that the volatile compounds produced by these microorganisms during growth can be detected by the e-nose and recognized according to a pattern model [17-18].

Off-flavours in foods originate mostly from bacterial and/or fungal metabolism and several studies have been carried out on the detection of the volatile chemicals produced from the microorganisms mentioned. The isolated species include gram-negative, gram-positive bacteria and several fungi [19-22]. The risk of fungal contamination is also related to several mycotoxigenic species that produce mycotoxins very harmful to human and animal health.

Therefore, early detection of the microorganisms offers many advantages for quality control in the foodstuffs industry. Similarly, specific volatile compounds have been identified and related to the growth of several microorganisms in biological samples and these results promise to be useful for medical diagnostics [23-26].

In this paper we review the results obtained by these instruments employed for the identification of microorganisms related to: i) infectious diseases, ii) food and beverage spoilage.

## **2. Sampling for detection and diagnosis of infection**

### *2.1 Clinical samples and rapid diagnosis*

Different sampling methods have been used for the volatile compounds detection in order to distinguish between normal and infected specimens and to see whether the pathogens might be detectable among other species. In the field of clinical microbiology, classical cultivation and isolation

methods are laborious and time consuming. The rapid screening of biological samples could allow faster and appropriate therapeutic treatment and would lead to a mortality rate decrease [23].

Consequently, several studies have been performed on biomedical specimens for rapid, near-patients diagnosis using e-noses. Table 1 provides a summary of microorganisms detected in clinical specimens by e-noses. In urinary tract infections e-noses were used to analyze urine samples directly or after short incubations in test tube systems containing complex media [27-29]. Moreover, a variety of data reduction and pattern recognition techniques are proposed to discern the species and/or different metabolic states of the bacteria [30-31]. In particular, it is reported that an e-nose was able to distinguish between uninfected and infected urine with *Escherichia coli*, *Proteus mirabilis* and *Staphylococcus* sp. [31].

**Table 1.** Application of the e-nose on biomedical samples for rapid microbial diagnosis.

Disease	Sample	Bacterial species detected* or screening for infection	Literature cited
Leg infections	Ulcers	Positive	[23]
Urinary tract infections	Urine	<i>Staphylococcus aureus</i> <i>Escherichia coli</i> <i>Proteus mirabilis</i>	[27-31]
Sinusitis Pneumonia	Breath	Positive	[32-34]
Tuberculosis	Sputum	<i>Mycobacterium tuberculosis</i> <i>Mycobacterium avium</i> <i>Pseudomonas aeruginosa</i>	[35]
Bacterial vaginosis	Swabs	Positive	[36-37]
Ear, nose, throat infections	Swabs	<i>Staphylococcus aureus</i> † <i>Pseudomonas aeruginosa</i> <i>Streptococcus pneumoniae</i> <i>Streptococcus pyogenes</i> <i>Haemophilus influenzae</i>	[38-40]

\* The samples were analysed using traditional cultural methods for confirmation.

† Including both methicillin-resistant and methicillin-susceptible.

Prospective studies have been performed to determine whether exhaled gas or breath analysis using an e-nose correlates with pneumonia scores or sinusitis. The results show good correlation with clinical diagnosis and provide new potential for diagnostic analysis [32-34].

Using an e-nose based on electroconductive polymers, Pavlou *et al.* examined the potential for the detection of *Mycobacterium tuberculosis* in sputum samples. These authors have shown, for the first time, that it is possible to discriminate effectively between human sputum samples infected with *M. tuberculosis*, *M. avium*, *Pseudomonas aeruginosa*, a mixed infection and the controls [35]. The

finding, although preliminary, is very encouraging and provides a rapid means to identify the organisms causing respiratory infections.

Finally, preliminary studies have also reported that e-nose can provide a promising means for detecting and identifying bacteria in vaginal, nasal, ear and throat swabs [36-40]. Further improvement of these studies could enable rapid diagnosis of bacterial vaginosis and upper respiratory infections.

## 2.2. Bacterial species and biochemical precursors

The studies described above are based on the development of new fast methods for monitoring bacteria vapours on the patient or directly in the clinical specimens. In other experimental work, artificial media have been employed to obtain from each microorganism biochemical pathways leading to the production of characteristic odours. In these experiments the microorganisms were previously isolated and inoculated in complex nutrient media. These studies explored the potential for discrimination between disease-causing pathogens and other isolates, by inducing biochemical precursors for the liberation of specific odours. The results showed that it is possible to discriminate between clinical anaerobic isolates of *Clostridium* sp. and *Bacteroides fragilis* collected from intra-abdominal infections [41]. Detection of microorganisms causing *Helicobacter pylori* infection [42] and other bacteria causing urinary tract infections was also achieved [31].

One of the aims of these experiments was also to provide a faster identification of the specific pathogens by reducing the incubation time of the cultures. Recently, a fast method to identify ten pathogenic organisms using an e-nose has been reported: these studies show that an incubation of 16 hours is sufficient to produce measurable volatile compounds [43]. Gardner *et al.* have also obtained important results by sampling bacteria such as *E. coli* and *S. aureus* during their growth phase. In this context, 100% of *S. aureus* were correctly classified during the first hour of incubation and in the lag phase [44-45]. Since the specific metabolites reflect the bacterial growth, it could be possible in the future to detect important vapours as markers of the different bacterial species. This could enable the development of specific sensors to obtain improvement in the discrimination patterns and rapid screening for diagnosis [46].

## 3. E-noses for detection of foods and beverage spoilage

In the food-processing industry quality assurance systems need to be rapid and range from organoleptic measurement to microbiological surveys. Generally, qualitative assessment of food spoilage is made by human sensory panels that evaluate air samples and discriminate which food products are good or unacceptable. Quantitative characterization is then made by gas chromatography and mass spectral analysis [47-48]. Compared to this methodology, the e-noses can perform odour detection continually and are not subject to individual sensitivity.

Bacterial contamination of food and drinks can generate unpleasant odours and toxic substances. Therefore, different industries are interested in the application of the e-nose both for monitoring of storage quality degradation and for detecting microbial contaminants.

One of the earliest reports of e-nose technology applied to food analysis concerned the vapours produced by microorganisms involved in sausage fermentation [49]. Subsequently, Arnold and Senter

reported that bacteria isolated from processed poultry were cultured to evaluate the profiles of volatile compounds generated and the e-nose was able to distinguish each of the isolated species [50].

Early detection of milk spoilage as well as different concentrations of spoilage bacteria and yeasts were also investigated. The results of these studies showed that, using an e-nose system, it could distinguish between volatile profiles of different species inoculated in milk-based media after two and five hours of incubation. Moreover, three bacterial concentrations of *Pseudomonas aureofaciens* ( $10^6$  cells/mL,  $3.5 \times 10^8$  cells/mL, and  $8 \times 10^8$  cells/mL) were clearly discriminated [51]. Although under development, these studies confirm the results of different works that explored the application of different gas sensors in the detection and identification of bacteria and moulds in milk and dairy products [19, 52-54].

Other studies, carried out to detect different microbial species (*Enterobacter aerogenes*, *E. coli*, *Pseudomonas aeruginosa*) inoculated in potable water, showed that  $10^2$  -  $10^4$  cells/mL is the lowest microbial concentration detectable by an e-nose [55].

As well as bacteria many fungal species were described to play an important role in the degradation of foodstuffs. Different species have been isolated from food and some studies have been performed on different fungal species isolated from cereal grain and from mouldy bread. The aims of these studies was to employ the e-noses for early detection and differentiation methods for fungal species prior to visible growth in cereal grains and bakery products [56-57]. The results indicate that sensor arrays could be used as rapid means for controlling and improving food product quality. Similar encouraging results were obtained in studies carried out on the detection of mycotoxigenic fungi in grains [58-59].

The studies described above analyzed gas mixtures from spoilage microorganisms previously isolated and inoculated in specific media (Table 2). A different approach is the direct detection of vapours in food samples. Subsequently, characterization of vapours by GC-MS was related to identification of contaminant microbes [60]. In these experiments, the e-nose is based on different gas sensors and the volatile compounds are estimated based on the individual sensor responses. All the results mentioned above are promising and further investigations to induce specific response patterns of the gas sensors may help both in the qualitative and quantitative analysis of food spoilage.

#### 4. Data analysis

Data processing techniques, used to perform detection, classification and description functions associated with the e-noses often include PCA, CA, Discriminant Function Analysis (DFA), Neural Network and Fuzzy Logic. Improvement and integration of new techniques are currently developing in order to enhance the accuracy of the prediction models [30, 61-63]. The systems optimizing knowledge extraction from e-nose are highly sophisticated and need to perform mathematical/statistical methods to implement and improve data analysis.

This application area is a very complex area of research in different fields such as medicine and biology, therefore, it is beyond the scope of this review to analyze data processing systems and to draw conclusions concerning new analytic models. Excellent works, which the reader is referred to, have

been published concerning this matter [16, 19, 64-67]. Table 3 lists e-noses and data processing methods referenced in this paper.

**Table 2.** Representative bacteria and fungi detected by e-nose and implicated in food spoilage.

Food or drink	Bacterial and fungal species**	Literature cited
Poultry	<i>Salmonella enteritidis</i> § <i>Escherichia coli</i> § <i>Listeria monocytogenes</i> § <i>Klebsiella pneumoniae</i> ‡ <i>Enterobacter cloacae</i> ‡ <i>Pseudomonas aeruginosa</i> ‡ <i>Acinetobacter calcoaceticus</i> ‡	[50]
Milk and dairy products	<i>Bacillus cereus</i> § <i>Pseudomonas aureofaciens</i> § <i>Pseudomonas fluorescens</i> § <i>Staphylococcus aureus</i> § <i>Candida pseudotropicalis</i> § <i>Kluyveromyces lactis</i> §	[19, 51-54]
Water	<i>Enterobacter aerogenes</i> § <i>Escherichia coli</i> § <i>Pseudomonas aeruginosa</i> §	[55]
Grains	<i>Fusarium moniliforme</i> ‡ <i>Fusarium proliferatum</i> ‡	[56, 58-59]
Bakery products	<i>Eurotium amstelodami</i> ‡ <i>Eurotium chevalieri</i> ‡ <i>Penicillium chrysogenum</i> ‡	[57]

\*\* The microbial species were obtained from several culture collections (§) or isolated (‡) from food/drinks and were used in cultures to detect volatile organic compounds.

## 5. Conclusions

In this review we have described the applications of electronic odour sensing systems for microbial detection in the fields of health care and food technology. Published literature is considerable and explores different experimental conditions to develop and implement these new analysis methods.

In our opinion, there are two problems associated with the e-nose technology. The first problem concerns the instrument performance with regards to physical parameters (e. g. sensitivity, selectivity, reproducibility, stability) and data treatment. These aspects are being continually improved for rapid and better characterization of a large number of odours [61, 68-70].

The second problem is related to the experimental procedures performed to obtain specific responses for odour detection and identification. For this purpose, different approaches are proposed

for sampling and providing specific information about the microorganism causative of disease or spoilage. The goals of these experiments are to identify the microbial species and/or to yield the chemical composition of the odours detected [71-75].

So far, the sensitivity of the human nose is far superior to that of any e-nose. Therefore, human panelists responses are still employed for odour classification. Solution to the listed problems and careful practical applications can provide rapid and useful tools in odours identification.

**Table 3.** Summary of commercially available e-noses, related sensors and data processing methods.

E-nose	Number of Sensors	Data processing method	References
MOSES II, Lenmartz Electronic, Tübingen, Germany	8 MOS, 8 QMB, 4 AGS	PCA	[17]
BH-114, Bloodhound Sensors Ltd, Leeds, UK	14 CP	PCA, DFA, CA, GA-NNs	[18, 22,31, 35, 41-42, 51, 55, 57-58]
Cyranose C320, Cyrano Sciences, Pasadena, CA, USA	32 CP	PCA, PLS, FCM, SOM, PNN, RBF	[26, 30, 33,38-40, 61]
FOX, 2000, Alpha MOS, Toulouse, France	6 MOS	PCA, NNs	[44-45]
Airsense Portable E-Nose (PEN2), Airsense Analytical, Schwerin, Germany	10 MOS	PCA, ANNs, k-NN	[43]
FreshSense, Bodvaki-Maritech, Kópavogur, Iceland	4 electrochemical for : CO, H <sub>2</sub> S, SO <sub>2</sub> , NH <sub>3</sub>	Statistical analysis, Duncan's multiple range	[60]
zNose™, Electronic Sensor Technology Newbury Park, CA, USA	1 SAW	Reference to calibration standards	[ 9-11, 71]

*Notes:* MOS: Metal-Oxide Semiconductors; QMB: Quartz Microbalance; AGS: Amperometric Gas Sensors; CP: Conducting Polymers; SAW: Surface Acoustic Wave. PCA: Principal Components Analysis; DFA: Discriminant Function Analysis; CA: Cluster Analysis; GA-NNs: Genetic Algorithms, back propagation Neural Networks; PLS: Partial Least Squares; FCM: Fuzzy C Means; SOM: Self Organizing Map; PNN: Probabilistic Neural Network; RBF: Radial Basis Function; NNs: Neural Networks; ANNs: Artificial Neural Networks; k-NN: k-Nearest Neighbour algorithm.

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