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Rapid Detection of Micro-Organisms in Sterile Body Fluids by Electronic Nose System

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Abstract: A recent development in odour sensing technology and artificial intelligence, as E-nose system, has rediscovered the use of smell in clinical diagnosis. Nowadays, this technology is becoming an interesting alternative for medical point-of-care devices. This study analyses the possibility of E-nose for rapid and accurate detection of micro-organisms in normal sterile body fluids, to ensure correct chemotherapy. In this study, 75 samples of different kinds of bacteria which are the major cause for Sepsis and Urinary Tract Infection were inoculated in 20 mL growth medium and incubated for 2 h for volatile generation. These samples were analysed with an electronic nose. The instrument, equipped with 12 Metal Oxide Semiconductor (MOS) sensors, was used to generate a pattern of the volatile compounds present in the pathological samples. The sensor responses were evaluated by Principal Component Analysis (PCA) and Artificial Neural Network (ANN). Good results were obtained in the classification of bacterial samples by using a neural network model based on a multilayer perceptron that learned using a back propagation algorithm. The methodology is simple, rapid and the results suggest that the electronic nose could be a used as a tool for detection.

Key words: Electronic nose, principle component analysis, artificial neural networks, bacterial species, control (growth medium)

INTRODUCTION

Disease can be defined as any condition where the normal functioning of body is impaired, leading to a change in normal state of health of an individual. One important group of diseases is known collectively as the infectious diseases which are caused by a large variety of pathogenic (Micro-organisms and Disease, 2006). Many of these micro-organisms have developed drug resistance which has made the treatment more difficult. They damage their host and if untreated, may eventually cause death. Among various specimens received by clinical laboratories, detection of micro-organisms in sterile body fluid has important diagnostic and therapeutic implications. These infections are often serious and a rapid diagnosis is required to ensure correct chemotherapy. The various sterile body fluids are blood, cerebrospinal fluid, peritoneal dialysis effluents and urine (Micro-organisms and Disease, 2006; Peter and Deidre, 2004). Among various kinds of infectious diseases in sterile body fluids, this research aims at rapid detection of most commonly prevailing micro-organisms causing infection in blood and urine which causes Sepsis and Urinary Track Infection.

MOTIVATION

India, with one of the world's largest populations, continues to struggle with extremely high infant and neonatal mortality rates. Neonatal infection (sepsis) now accounts for 50% of deaths among community-born (and 20% of mortality among hospital-born) infants. Invasive bacterial infections encompass clinical diagnoses of septicemia, pneumonia and meningitis. Together, these infections are termed neonatal sepsis and account for over half of the newborn deaths at the district and sub-district level in India. Sepsis is the most common (80-90%) primary diagnosis for admission in Indian hospitals (NICHD, 2006).

Urinary tract infection is a dangerous and unrecognized forerunner of systemic sepsis. Urinary tract

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infection is another important cause of morbidity and mortality in Indian subjects, affecting all age groups across the life span. Though *Escherichia coli*, which is normally present in the gastrointestinal tract, is the commonest causative organism, other gram negative colonic bacteria have been gaining prominence in India over the last two decades. Because of the proximity of the gut to the urinary tract, these organisms ascend through the urinary passage to the urinary bladder and the kidneys to produce infection (Acharya, 1992).

Thus rapid detection and identification of these two diseases gain more importance. Nowadays in the filed of clinical microbiology, current techniques generally require 24-48 h to identify and characterise a pathogenic micro-organism following a series of biochemical tests. In order to overcome these difficulties and to improve the method of detection for rapid diagnosis, electronic nose system is employed in this research.

ELECTRONIC NOSE (E-NOSE)

An Electronic nose (E-nose) is an analytical device used for detecting vapour chemicals. It consists of broadly tuned sensory array which interacts with a broad range of chemicals with varying strengths (Gibson *et al*., 2000). Consequently, an incoming analyte stimulates many of the sensors in an array and elicits a characteristic response pattern. These patterns are then further analysed for the benefit of a specific application.

Over the last 20 years, due to the development of chemical sensor system, odour analyses made possible and in the past few years various research works have been experimenting with electronic noses in the detection of micro-organisms (Gardner, 2006; Persued, 2005; Gardner, 2000; Pavlou, 2002; Ritaban, 2002). It is a logical progression to look at the incidence of infection by micro-organisms using changes in the smell of the patients themselves or the odour of clinical samples (Gibson *et al*., 2000). This technology aims at mimicking the mammalian sense of smell by producing a composite response unique to each odorant.

Further to this idea, the combination of selective culturing techniques currently used and the subsequent measurement of the odours generated could be used to identify the causative organisms in clinical infections more quickly. The following Table 1 gives the volatile compound liberated by different bacterial species (Gardner, 2000; Kodogiannis, 2002).

Objective: This study describes and analyses the applications of E-nose system for microbial detection in the field of medicine, where fast detection methods are essential for appropriate management of health care.

Table 1: Generation of microbial volatiles due to metabolic reaction with specific biochemical precursors

Bacterial sp.	Medium	Volatile compound
E.coli, *Klebsiella* sp.	Arabinose, Lactose	Ethanol
Proteus sp., *Klebsiella* sp., Staph.Aureus, *Pseudomonas* sp.	Tryticasesoy broth	Isobutanol, Isopentyl acetate ketones
Proteus sp., Enterococus, *Klebsiella* sp.	Acetylcholine	Trimethylamine, ethyl acetate
Proteus sp.	L-methionine	Dimethyl sulphide, methyl mercaptan
P.aerugiuoss	Broth complex	Isobutylamine, Isopentylamine, ethylamine

Thus the objectives of this study are to

- Make odour fingerprints between medical samples of healthy and infected.
- Develop classification model using statistical methods and also using soft computing techniques
- To compare the results for discriminating the infected with healthy.

MATERIALS AND METHODS

Apparatus: The E-Nose used in this research is based on αFOX 3,000 (Alpha MOS Sensory Array) and comprises of odour sensor array. This sensor array consists of 12 sensors (Alpha MOS, France) and these are specially designed for food analysis at Central Food Technical Research Institute (CFTRI), Mysore, India.

The principle of operation of áFOX is based on a Metal Oxide gas sensor array which, due to specific reactions to various kinds of molecules, produces an olfactive picture or finger print from an odour. Metal Oxide Sensors (MOS) devices are based on doped or undoped metal oxides. The principle of detection is based on conductivity measurements, in the presence of a combustible gas or odour, the oxygen species which are adsorbed on the metal oxide react and are removed from the surface. As a result, the conductivity of the metal oxide film changes. The different parameters are processed using statistical methods to give a 'digital print' and thus identify the bacteria producing the odour.

The sample is contained into a sealed vial, where the headspace is finally taken using a gas syringe and injected into this specially adapted E-nose.

Samples: The bacterial samples used in this study are among most common bacterial pathogens causing UTI/Sepsis i.e. Escherichia coli, Staphylococcus aureus,

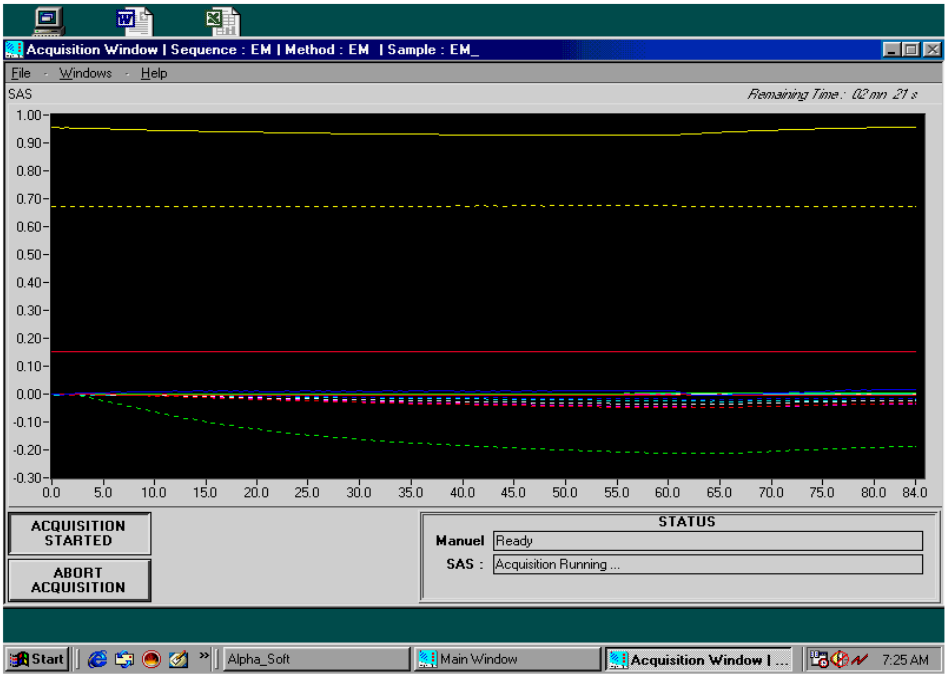

Fig. 1: Change of resistance for empty head space

Pseudomonas aeruginosa and Citrobacter. These samples where collected from JSS hospital, Mysore.

Operative procedure: A suspension of each bacteria culture, equivalent to 0.5 McFarland turbodometer Standard, which is equal to 10^5 cells mL^{-1}, was made using sterile 0.15M NaCl. One milliliter of the suspension was aseptically transferred into a 200 mL sterile bottle that contained 20 mL of Brain Heart Infusion Broth (BHIB).The inoculated bottles, along with the non-inoculated (control) bottles, were incubated at 37°C for 2h to attain log phase

The 20 mL of sample is contained into a sealed vial; the headspace is finally taken from the vial using a gas syringe and injected into the Enose.

Data analysis: The software used for data acquisition is αFOX 3000 software. The following parameters were set before the samples are subjected to analysis by E-nose system (Alpha MOS). Acquisition time: 12sec, Acquisition period: 0.5sec, Delay: 100 sec, Flow: 150 mL min^{-1}, Headspace Generation: 120 sec and base line recovery period for 10 min. Air is passed over the pathogen sample and passed into Enose. The change in resistance for empty headspace and different samples are as shown in the Fig. 1-3.

The data obtained from the sensor array for the 75 samples were analysed by Principal Component Analysis (PCA) performed with Alpha Soft (v. 7.0 Alpha MOS, France) and Artificial Neural Network (ANN) using Neuro-Solutions software (v. 4.2 Neuro Dimension Inc.,Gainesville, Florida, USA).

Data were collected as follows: Totally 15 pathogenic samples of different species with same conditions were subjected for analysis. The operation was repeated five times for each one so that totally 75 set of readings were obtained. All data were normalized using a fractional difference model: dr = (R-R0)/R0 where R is the response of the system to the sample gas and R0 is the baseline reading, the reference gas being the ambient room air. Thus totally a matrix of 1360 data items was constructed.

Principal Component Analysis (PCA): Principal Components Analysis was used for explorative data analysis as it identifies orthogonal directions of maximum variance in the original data, in decreasing order and projects the data into a lower-dimensionality space formed of a subset of the highest-variance components. The orthogonal directions are linear combinations (Principal Components PCs) of the original variables and each component explains in turn a part of the total variance of the data. In particular, the first significant component explains the largest percentage of the total variance, the second one, the second largest percentage and so forth (Fig. 4).

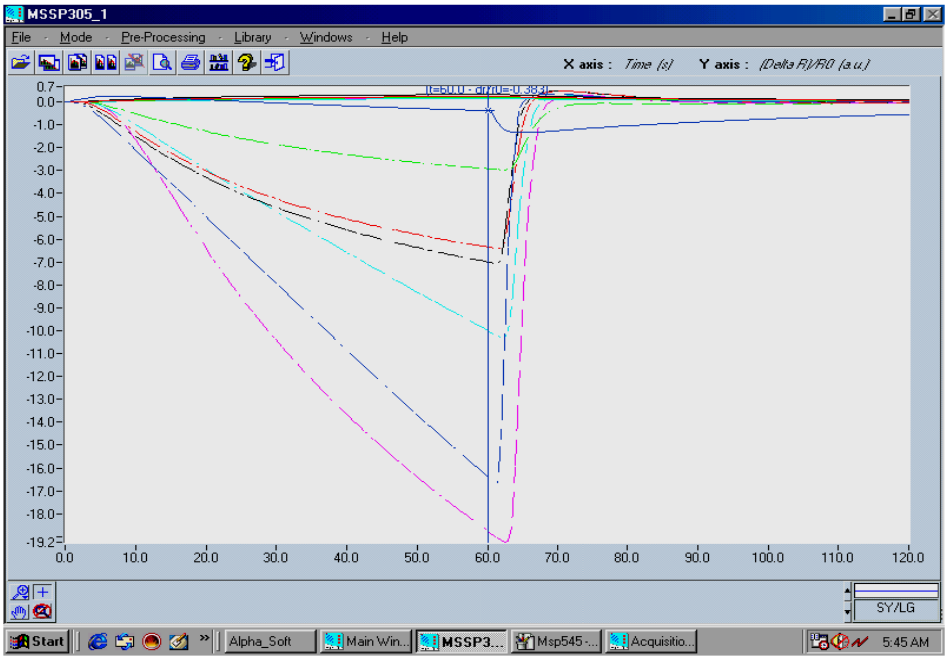

Fig. 2: Change of resistance for sample pseudomonas headspace

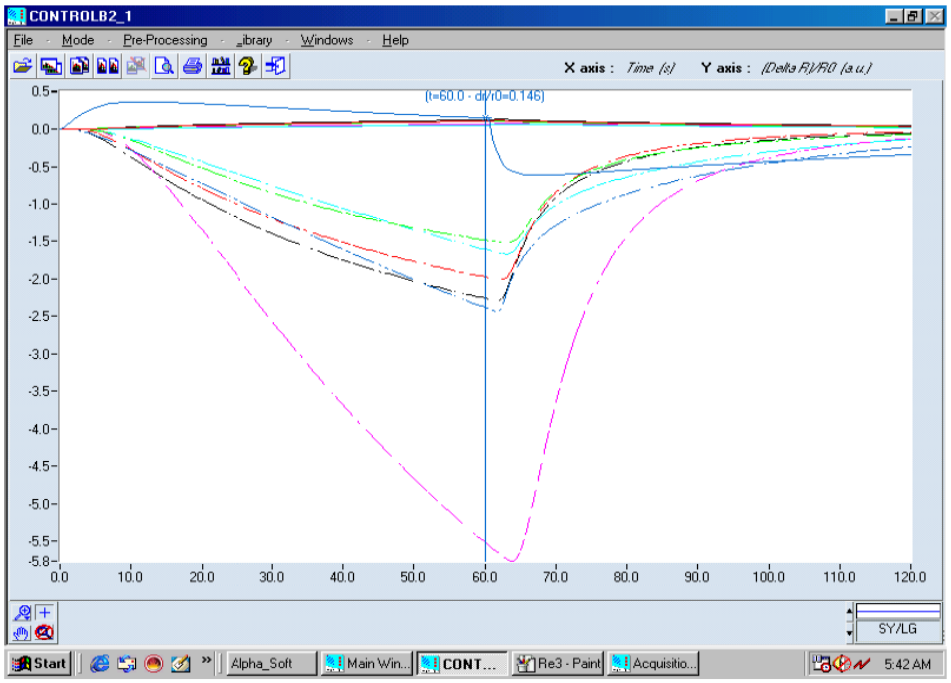

Fig. 3: Change of resistance for sample control (growth medium) headspace

After PCA classification it is evident that there is discrimination between different kinds of bacteria and also between normal and infected. Hence the data was further analysed by Artificial Neural Network (ANN).

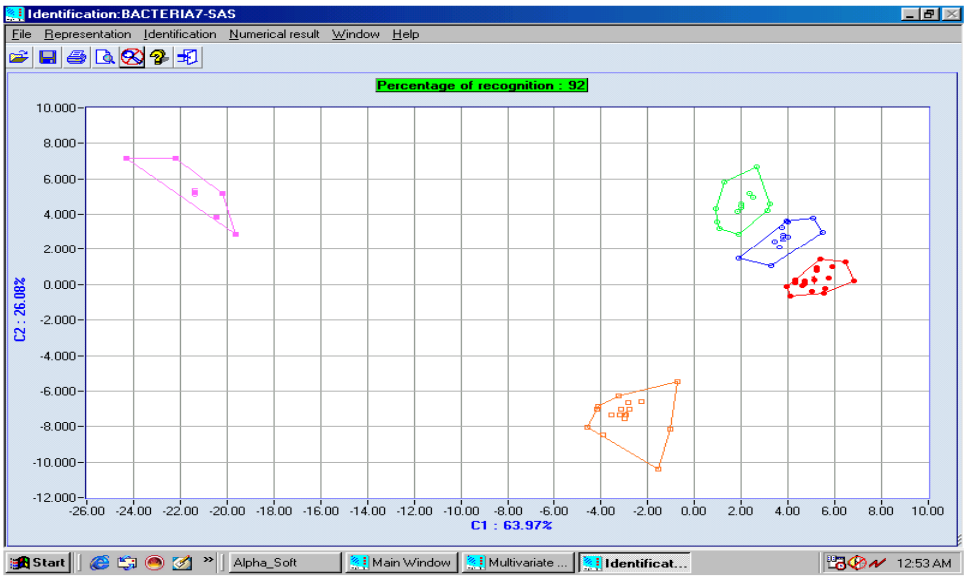

Fig. 4: Discrimination of type bacteria by PCA- citrobacter, staphylococcus, pseudomonas, ecoli, control (growth medium)

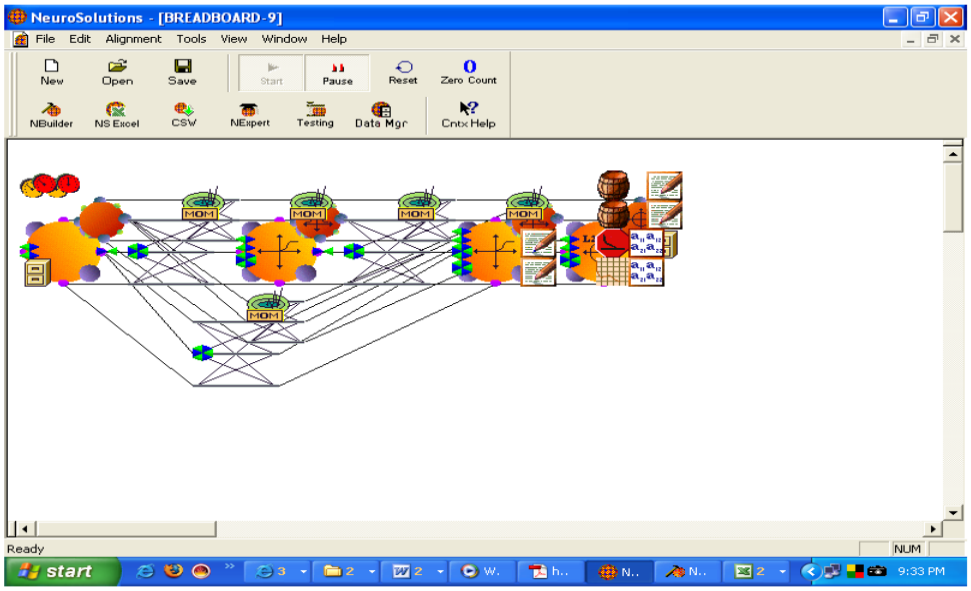

Fig. 5: Architecture of ANN for bacterial classification

Artificial Neural Networks (ANN): Neural networks learn from examples through iteration, without requiring a priori knowledge of the relationship among variables under investigation. The neural network model used in this study was a Multilayer Perceptron (MLP) that learns using an algorithm called back propagation with an adaptive learning rate and momentum of 0.01 and 0.70, respectively. The network was composed by 23 input neurons, a one-hidden-layer net with 4 hidden units in the hidden layer and 5 neurons in the output layer (one for each sample type). Sensor responses of medical samples were repeatedly fed into the net and training was

Table 2: General performance of the ANN for 10 bacterial samples in the testing set analysed based on the processing the data from 12 sensors

Performance	Groups
MSE	0.38312887
NMSE	0.125613516
MAE	0.296738632
Min Abs error	0.063832451
Max Abs error	1.129843017
r	0.953341976

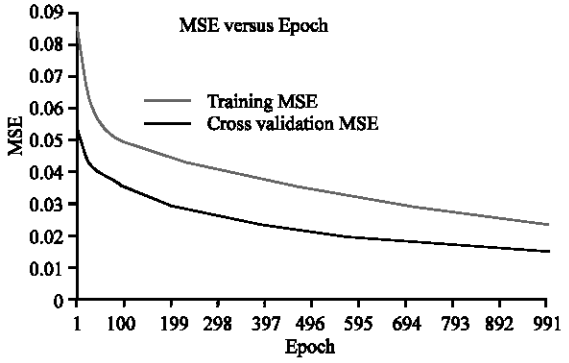

Fig. 6: General MSE vs Epoch performance of the ANN for 10 bacterial samples in the testing set analysed based on the processing the data from 12 sensors

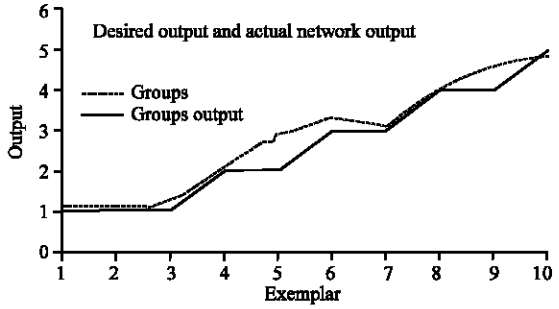

Fig. 7: Desired output vs actual for 10 bacterial samples in the testing set

accomplished by automatically adjusting the connection weights in order to reduce the error level. The architecture of ANN for this analysis is given below in Fig. 5.

The ANN training was terminated using the method of stopping with cross validation. Training was then stopped at the point of the smallest error in the validation set. This allowed avoiding overtraining. Sixty percent of the randomised sample data set were used as the training set (56 samples), 12% as the cross validation set (9 samples) and 13% as the testing set (10 samples). Since a neural network can arrive at different solutions for the same data, the network was trained several times with different values of initial network weights. The goal was to try and find a neural network model for which multiple training approaches the same final Mean Squared Error

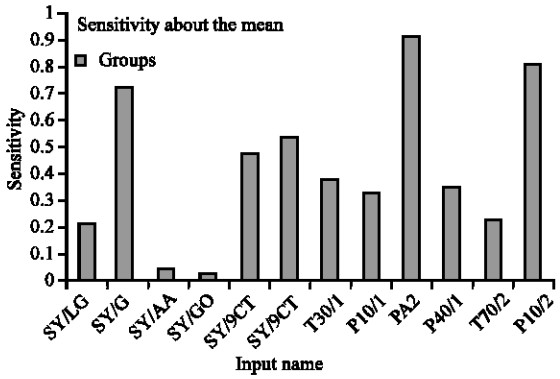

Fig. 8: Sensitivity report of the sensor responses for the 10 bacterial samples in the testing set

(MSE). The goal was met with the following performance parameters as given below as Table 2 and Fig. 6 and 7.

MSE-Mean Square Error, NMSE-Normalised Mean Square Error, MAE-Mean Absolute Error, r-Correlation Co-efficient.

In order to know the effect that each input had on the network output, a sensitivity analysis was performed. This allowed to identify and to eliminate irrelevant inputs and to produce a smaller network with better generalization as shown in Fig. 8.

RESULTS AND DISCUSSION

The cumulative variance explained by the first two principal components was 98.23 and 1.03%. The first two principal components clearly separated the Citrobacter and Staphylococcus from the three others. The third component explained 0.61%of the total variance. Ecoli and Control are not distinctly separated. This may be due the kind of sensors used for this research. Thus choice of appropriate sensors is critical for detection of microbes in samples.

Once explored the sensor data by principal component analysis and determined that the five different kinds of bacteria could be separated, a classification model was performed by the ANN technique.

In this study, all responses from the electronic nose sensors were used as inputs to the neural network that was built. The relative importance of each input varied considerably as illustrated by the reports of the sensitivity analysis as shown in Fig. 2. Only a few sensors were able to classify the medical samples and these were: 1SY/G,SY/gCT,Sy/gCT1,T30/1,P10/1,p10/2,P40/1,PA2 (identified by the instrument manufacture). With the array dimension reduced, the subsequent neural networks, consisting of 8 inputs and 5 outputs, is to be built and trained in future. The results of ANN analysis so obtained can be visualized by a table showing the general

performance indexes of the network. Every sample type was correctly classified by the network as Pseudomonas, *E. coli*, Control.

Volatile and semi-volatile organic compounds present in the headspace aroma create different odour prints which contribute significantly to classify the micro organisms so that it can be discriminated from the healthy sample. The results suggest that the electronic nose could be a useful tool for screening bacterial volatile compounds and for characterizing different kinds of bacteria. Furthermore, the proposed methodology is simple, rapid and does not require isolation of the volatile components. This makes the technique particularly useful for rapid detection of micro organisms which can help in saving human life to great extent.

In this research, e-nose with metal oxide semi conductors is employed which can be further extended by using other e-nose sensor technology such as Piezoelectric sbased sensors-Bulk Acoustic Wave Sensors (BAW) and Sound Acoustic Wave Sensors (BAW), Conducting Polymers, Optical transducers etc. The sensory data's are analysed by the classification model using Back propagation network which can be replaced by other soft computing technique like GANN, PCANN, Neuro Fuzzy.

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