Effectiveness of an Electronic Nose for Monitoring Bacterial and Fungal Growth

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Abstract. Growth of microbial organisms such as bacteria and fungi generates volatile organic compounds and fixed gases. An electronic nose consisting of 15 metal-oxide sensors (NC State E-Nose) was used to detect and classify bacteria and fungi. Three preliminary experiments were conducted with the electronic nose using odorous stimuli related to microbial contamination. The results suggested that the NC State E-Nose could classify bacteria, fungi, and associated volatile organic compounds. A further experiment was performed to detect and classify five fungi commonly found in indoor environments. These fungi were *Aspergillus flavus, Aspergillus niger, Penicillium chrysogenum, Cladosporium cladosporioides, and Stachybotrys chartarum.* The fungi were cultured on two types of media, Potato Dextrose Agar (PDA) and Czapek-Dox Agar. The NC State E-nose was capable of discriminating among these fungi with up to 96% accuracy.

1. Introduction

Growth of bacteria and fungi on organic matter generates a broad range of volatile organic compounds and fixed gases. Wessén and Schoeps [1] and Sunesson et al. [2] showed that the presence of certain volatile organic compounds can be used as an indicator of the presence and identity of microorganisms. Holmberg [3], in a dissertation at Linköping University in Sweden, used an electronic nose with 15 sensors to classify 5 types of bacteria (*Escherichia coli, Enterococci* sp., *Proteus mirabilis, Pseudomonas aeruginosa, and Staphylococcus saprophyticus*). The 15 sensors included 9 MOSFETs, 4 Taguchi type sensors, 1 carbon dioxide sensor, and 1 oxygen monitor. The volatile compounds generated by the bacteria were sampled from agar plates. The results suggested that this E-nose could successfully classify *Escherichia coli* and *Enterococci* sp. but was less successful with the other bacteria.

Gardner et al. [4] used an electronic nose that contained six commercial metal oxide sensors, a temperature sensor, and a humidity sensor to predict the class and growth phase of two types of bacteria, *Escherichia coli and Staphylococcus aureus.* The six sensors were designed to

detect hydrocarbons, alcohols, aldehydes/heteroatoms, polar molecules, and nonpolar compounds. The best mathematic model identified 100% of the unknown *S. aureus* samples and 92% of the unknown *E. coli* samples.

Other studies have also found that bacteria can be discriminated using an electronic nose. In an evalutation of 7 bacterial strains, Vernat-Rossi et al. [5] were able to correctly discriminate 98% of a training set with a cross-validation estimate (test set) of 86% using 6 semiconductor gas sensors. Parry et al. [6] found that swabs from chronic venous leg ulcers with haemolytic streptococci could be discriminated from those without bacteria using an electronic nose with polymer sensors. Studies at AromaScan PLC [unpublished data from Dr. Krishna Persaud] showed that polymer sensors performed well in discriminating multiple samples of 5 different types of bacteria.

Keshri et al. [7] used an electronic nose consisting of 14 polymer sensors to classify six spoilage fungi (four *Eurotium* sp., a *Penicillium* sp. and a *Wallemia* sp.). The headspace was sampled after 24, 48, and 72 hours of growth. The electronic nose discriminated the fungi at the 24-hour mark (prior to visible signs) with an accuracy of 93%. The best results occurred at the 72-hour mark.

2. Objectives of the present study

The purpose of the present study was to extend our current understanding of the capacity of an electronic nose to indentify and classify microorganisms including bacteria and fungi. When conditions are favorable and a nutrition source is present, microbial organisms such as fungi and bacteria can grow almost anywhere. Microorganisms have been shown to generate volatile organic (VOCs) while metabolizing nutrients, and these VOCs have been used as indicators of microbial growth. Microorganisms not only generate airborne contamination in the form of VOCs, but also generate toxins and propagules including conidia (spores) and bacterial cells. When microoganisms infest buildings, they can create a potentially hazardous environment. Individuals exposed to environments containing high concentrations of airborne contaminants from microbial organisms report symptoms including eye and sinus irritation, headaches, nausea, fatigue, congestion, sore throat, and even toxic poisoning. Sick Building Syndrome (SBS), which includes health symptoms arising from poor indoor air quality, have been correlated with the presence of fungi [8]. A study of two housholds reporting indoor environmental complaints correlated the presence of excessive VOC's with the presence of fungal contamination [9]. Typical, signs of microbial contamination include water damage, high levels of humidity, and physical presence. However, these signs are not always present and therefore cannot be used as sole indicators of microbial contamination.

Current methods for detecting microbial contamination include visual inspection, air and material sampling with culture analysis, and air sampling coupled with gas chromatography/mass spectrometry [10,11]. These methods, however, can be inconclusive, time consuming, and expensive. There is a need for rapid detection of the presence of microbial contamination in order to minimize its impact. The studies described in this paper indicate that the use of an electronic nose can reduce the time required to detect, discriminate, and identify microbial contamination.

3. NC State electronic nose

An electronic nose instrument was designed and constructed at North Carolina State University [12,13,14] that uses an array of metal oxide sensors for measuring odor in air samples. It consists of a sampling unit, a sensor array, and a signal processing system. The sampling unit, which consists of a pump and a mass flow controller, directs the air sample containing the odorant under investigation across the sensor array. The current configuration allows for sampling from a set of 12 odorants, a reference sample (filtered odorless dry ambient air), and a washing agent (ambient air bubbled through a 2% butanol solution). The sensor array is comprised of 15 different metal oxide sensors each producing a different response pattern for the odorant under investigation. Twelve of the 15 metal oxide sensors are manufactured by Capteur (Didcot, UK) and include sensors for isopropyl alcohol, toluene, hydrogen sulfide, nitrogen dioxide, chlorine, butane, propane, hydrogen, carbon monoxide, heptane, ozone and general VOCs. The remaining three metal oxide sensors are produced by Figaro USA (Glenview, IL) and include a methane, a combustible gas, and a general air contaminant sensor. The overall combination of the response patterns defines the odorant under investigation. All of the sensor response patterns are digitized and recorded using a National Instruments® Data Acquisition Card controlled by LabVIEW®. The data are analyzed with MATLAB® using signal processing algorithms developed by Kermani [12] and Gutierrez-Osuna [13].

The raw data are first compressed using windowing functions that produced a set of four features for each sensor. Linear discriminant analysis (LDA) is then applied to the compressed data to maximize class separability. Sixty percent of the compressed data is randomly selected to form a training set for the classification algorithms. K nearest neighbors (KNN) and least squares analysis (LS) are both employed to classify the remaining 40% of the compressed data [15]. This method is performed a hundred times and the average score is used for the final classification score.

4. Preliminary experiments with NC State electronic nose

Three preliminary studies of the effectiveness of the NC State E-Nose were conducted on odorous stimuli related to microbial contamination. These studies included: 1) discrimination among ten bacterial organisms, 2) discrimination among five volatile organic compounds known to be generated during fungal and bacterial growth, and 3) discrimination among three fungal organisms. These studies described below showed that the NC State E-Nose is effective in discriminating odors associated with microbial contaminants. This prompted a final experiment in which the three fungal organisms from the second preliminary experiment along with two other fungi and two controls were investigated.

4.1. *Preliminary bacterial experiments*

Ten bacterial standards were grown on agar using the Prompt™ inoculation system (Becton Dickinson), and the headspace was sampled from the petri dishes 24 hours after inoculation. The ten bacteria included: *Escherichia coli, Enterobacter cloacae, Group D enterococcus, Citrobacter freundii, Klebsiella pneumoniae, Staphylococcus agglutinin negative,*

Staphylococcus aureus, Serratia fonticola, Proteus mirabilis, and Pseudomonas aeruginosa. The stimuli were sampled for 60 seconds with a 30 second wash cycle followed by a 240 second reference sample. The best classification algorithm yielded a correct percent classification of 82.5% (using leave-one-out cross-validation). The confusion matrix for these bacteria is given in Table 1. The statitical technique of linear discriminant analysis (LDA) was applied to the compressed data to maximize class separability followed by a K Nearest Neighbor classifier. The scatter plot generated by LDA is given in Figure 1. The LDA scatter plot suggests very good separation between classes, but the 82.5% validation rate indicates that the classifier overfits the training data, since only 4 sniffs per culture were collected. This prompted us to collect a larger data set for the final experiment reported in section 5 of this article.

Table 1: Confusion matrix from bacteria with percentages of classification

	True Class									
Predicted Class	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1 *Escherichia coli*	75	0	0	0	0	0	0	0	0	0
2 *Enterobacter cloacae*	0	100	0	0	0	0	0	0	0	0
3 *Group D enterococcus*	25	0	50	0	0	50	0	0	0	0
4 *Citrobacter freundii*	0	0	0	75	25	0	0	0	0	0
5 *Klebsiella pneumoniae*	0	0	0	25	75	0	0	0	0	0
6 *Staphylococcus agglutinin negative*	0	0	50	0	0	50	0	0	0	0
7 *Staphylococcus aureus*	0	0	0	0	0	0	100	0	0	0
8 *Serratia fonticola*	0	0	0	0	0	0	0	100	0	0
9 *Proteus mirabilis*	0	0	0	0	0	0	0	0	100	0
10 *Pseudomonas aeruginosa*	0	0	0	0	0	0	0	0	0	100

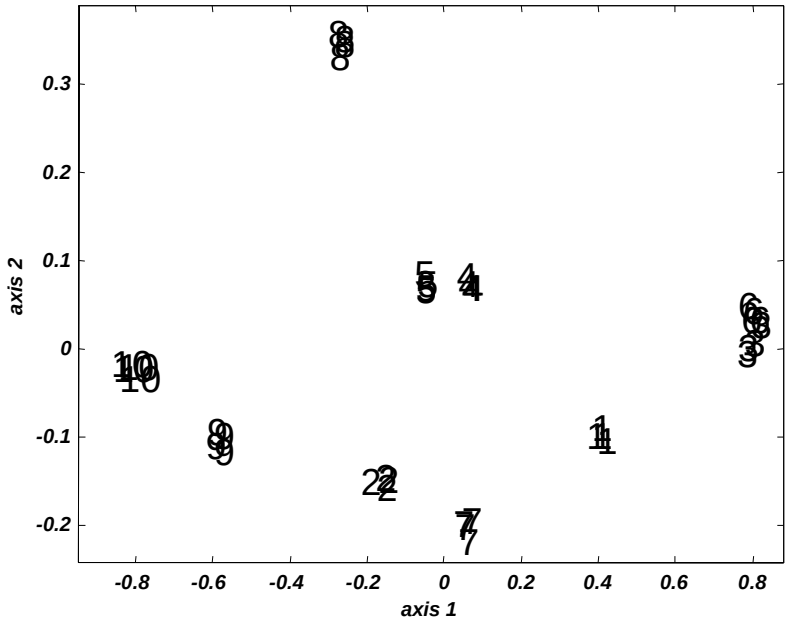

Figure 1: LDA scatter plot of bacterial data

4.2 *Preliminary volatile organic compound experiments*

In the second preliminary experiment the sensitivity of the NC State E-Nose to three VOCs generated by fungi was evaluated. Three VOCs selected for the experiment were ethanol, 3-pentanone, and 2-methyl-1-propanol. Serial dilutions of each chemical were prepared with dionized water to obtain eight concentrations. The headspace of each concentration was randomly sampled 3 times daily for 5 days from 30 ml amber bottles containing a 15 ml of solution. The headspace was drawn through a small hole in the lid of the bottle using a PVC tube. The results from the preliminary experiments on the volatile organic compounds indicated that detection thresholds for the NC State E-Nose were between 0.003% and 0.002% for the sample dilutions (see Table 2). The percent correct classification for all the chemical samples (and the diluent control) into their respective chemical classes was obtained by training a separate classifier at each concentration (see Table 3). These data suggest that the NC State E-Nose is capable of detecting and discriminating volatile compounds known to be generated during fungal growth.

4.3 *Preliminary fungal experiments*

Three fungi, *Aspergillus flavus, Aspergillus niger,* and *Penicillium chrysogenum*, were incubated at 28°C on 100 mm petri dishes containing Potato Dextrous Agar (PDA). The headspace above each fungus was sampled through a small hole in the center of the lid of the petri dish and an inline one micron filter for removing conidia (spores). The percent correct classification for the three fungi continued to improve through the seventh day of growth (see Table 4). It can be seen that 90% classification was obtained using KNN on days five and seven combined. The conclusions from this and the other two preliminary experiments indicated that the NC State E-Nose is capable of discriminating odors generated by microbial samples.

Table 2: Percent correct detection of the headspace samples from the serial dilutions

Chemical Type	Classification Method	Percent Concentration of Serial Dilution				
		0.050%	0.013%	0.006%	0.003%	0.002%
Ethanol	KNN	99%	95%	97%	88%	74%
	LS	99%	95%	97%	88%	74%
2-Methyl-1-Propanol	KNN	99%	92%	84%	81%	72%
	LS	99%	92%	83%	82%	72%
3-Pentanone	KNN	100%	99%	99%	98%	85%
	LSS	100%	99%	99%	98%	85%

Table 3: Percent correct discrimination of headspace samples of 3 VOCs and a diluent control for each serial dilution

Classification Method	Percent Concentration of Serial Dilution				
	0.050%	0.013%	0.006%	0.003%	0.002%
KNN	97%	94%	84%	84%	76%
LS	92%	83%	73%	75%	72%

Table 4: Percent correct classification for 3 fungal species grown on PDA

Classification Method	Day of Growth					
	0	1	3	5	7	5&7
KNN	38%	39%	69%	75%	79%	90%
LS	39%	39%	56%	69%	79%	82%

5. Final experiment with NC State electronic nose

An additional experiment was performed on a larger data set of fungi with improved instrumentation and sampling techniques. Five fungi (*Aspergillus flavus, Aspergillus niger, Penicillium chrysogenum, Cladosporium cladosporioides, and Stachybotrys chartarum)* were incubated at 28°C on 150 mm petri dishes containing Potato Dextrous Agar (PDA), a complex media rich in nutrients, and Czapek-Dox Agar (CZ), a minimal media. These two types of media were used in order to provide two different growth environments and to produce different growth rates (see Figure 2). Twenty four petri dishes of each media were inoculated with 0.5 ml of an individual spore suspension containing 10,000 condia/ml from each fungus respectively. The suspensions were prepared using a Spencer hemacytometer with improved Neubauer ruling. An autosampler was constructed to sample uniform air volumes at controlled intervals. Using the autosampler, air samples from the headspace of each petri dish containing one species on each media were randomly sampled ten times each after 24 hours and every other day thereafter for two weeks. The headspace above each fungus was sampled through a small hole in the center of the lid of the petri dish using a PVC tube and an inline two micron filter for removing conidia (spores).

The data were analyzed using two classification protocols. In the first protocol, the data were grouped into 12 classes: 5 fungal species grown on PDA and CZ respectively plus 2 controls (the two media PDA and CZ without fungal growth). The results are shown in Table 5. After 24 hours of growth, the percent classification was 90% for K nearest neighbors, and 76% for least squares. Classification for 12 classes reached a maximum after 5 days of growth, with an accuracy of 96% for K nearest neighbors and 94% for least squares. After day 5, the percent classification began to decrease slowly. By day 15, the percent classification was reduced to 89% for K nearest neighbors and 69% for least squares.

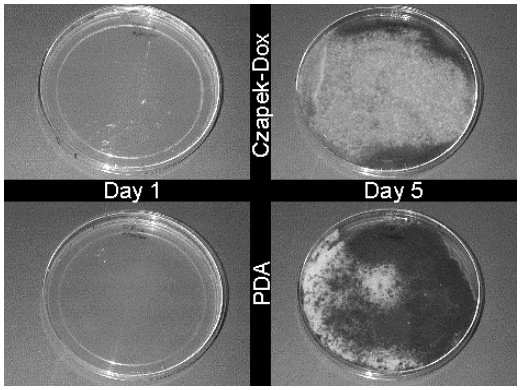

Figure 2 *Stachybotrys chartarum* growing on PDA and Czapek-Dox at day 1 and 5

Table 5: Percent classification for 12 classes (5 fungal species on two different media and 2 control media)

Classification Method	Day of Growth							
	1	**3**	**5**	**7**	**9**	**11**	**13**	**15**
KNN	90%	91%	96%	94%	89%	93%	93%	89%
LS	76%	90%	94%	90%	93%	86%	80%	69%

Table 6: Percent classification of 7 classes (5 fungal species and two control media)

Classification Method	Day of Growth							
	1	**3**	**5**	**7**	**9**	**11**	**13**	**15**
KNN	89%	90%	94%	93%	89%	94%	94%	92%
LS	79%	88%	93%	91%	95%	90%	92%	86%

In the second classification protocol, the data were grouped into 7 classes: 5 fungal species (independent of media used for growth) plus 2 controls (the two media PDA and CZ without fungal growth). In other words, each of the fungi grown in PDA and CZ were combined into a single class. After 24 hours of growth, the percent classification was 89% for K nearest neighbors, and 79% for least squares. Classification reached a maximum after 5 days of growth, with an accuracy of 94% for K nearest neighbors and 93% for least squares. After day 5, the percent classification oscillated around an average percent classification of 92% with a standard

deviation of 2%. The results are shown in Table 6. This final experiment using 5 fungi resulted in improved classification beyond that obtained for 3 fungi in the preliminary results.

6. Conclusions

Both the preliminary studies as well as the final experiment with 5 fungi showed that the NC State E-nose can detect and classify microorganisms on the basis of volatile emissions. The improved classification in the final experiment with fungi was independent of the media used to grow the fungi. Furthermore, correct classification was achieved with the NC State E-Nose at 24 hours of growth. Thus the NC State E-Nose has potential to be used for early detection of microbial contamination.

7. References

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