

E-nose biosensors for detection of plant diseases

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Abstract

Electronic nose (e-nose) biosensor is type of volatile sensing device that has wide range of application in food industry in spoilage detection and flavor analysis, detection of pollutants in the environment and disease diagnosis. It combines the principles of an electronic nose with biosensing technologies. They are frequently being used to acquire the real-time information about physical and chemical nature as well as quality of plant.

Introduction

Electronic nose (E-nose) is an artificial sensing technology comprised of a range of partially selective gas sensors which imitates the human olfactory system. The E-nose system considers the total volatiles emitted from samples to generate a digital fingerprint using appropriate statistical tools to discriminate multifarious smells. The term “electronic nose” was coined in 1988 by Gardner and Bartlett, who later defined it as “an instrument which comprises an array of electronic chemical sensors with partial specificity and appropriate pattern recognition system, capable of recognizing simple or complex odors and eliminates operator fatigue and enables the detection and categorization of mixture of scents. The sensor technology of artificial olfaction had its beginnings with the invention of the first gas multisensor array in 1982. The standard components of an electronic nose system are multisensor array, an information-processing device like an artificial neural network (ANN), software with digital pattern-recognition algorithms, and aroma reference-library databases having stored files with digital fingerprints of specific aroma reference patterns (Wilson and Baietto 2009).

Biosensor

A biosensor is an analytical device that combines a biological component (such as enzymes, antibodies, or whole cells) with a physicochemical detector to detect a specific analyte. Biosensors are often used for detecting substances like glucose, pathogens, toxins, or environmental pollutants.



E-nose Biosensor

An e-nose biosensor integrates the principles of both electronic noses and biosensors. It typically uses biological sensing elements (like enzymes or antibodies) that interacts with specific analytes (such as specific volatile compounds or biomolecules). The interaction produces a measurable signal (like electrical or optical) that can be detected by the electronic nose component of the device.

Emission of volatile organic compounds (VOCs) by plants

When plants are infected or under stress from diseases such as fungal infections or bacterial pathogens, they emit specific volatile organic compounds (VOCs). These VOCs can serve as biomarkers indicative of the presence of disease. These are easily vaporized, and are accessible for detection within sampled air by the e-nose biosensor. These VOCs can be grabbed by the sensor array of the E-nose setup to generate a fingerprint and discriminate between non-affected and affected plants or even among different types of diseases. Numerous artificial nose prototypes have been created to distinguish between various complicated vapor mixes that comprise a wide variety of VOCs. All of these prototypes together show distinct approaches to electronic aroma detection, using different kinds of gas sensors such as optical, surface acoustic wave, bulk acoustic wave, metal-oxide semiconductive polymers, conductive electroactive polymers, electrochemical, micro-electromechanical system, fluorescence, catalytic field-effect, metal oxide semiconductor, complementary metal oxide semiconductors, metal oxide semiconductor field effect transistor, quartz crystal microbalance and surface acoustic wave sensors.

E-nose instrumentation

The basic components of an e-nose biosensor are as follows:

1. An aroma delivery system: - to transfer the volatile aromatic molecules from the source material to sensor array system.
2. A chamber where sensors are placed: - the temperature and humidity are maintained at a fixed level which otherwise affect the aroma molecules adsorption. The sensor array can detect and measure the complex mixture of VOCs emitted by plants. They generate a unique pattern or fingerprint based on composition and concentration of these compounds.
3. An electronic transistor which transforms chemical signal into electrical signal and amplifies it.
4. A digital converter that converts signal from electrical to digital.
5. A computer microprocessor is required to read the digital signal and display the output which is then statistically analyzed for sample classification or recognition is carried out.

Applications of E-nose biosensors for plant disease diagnosis

Plant pathogenic bacteria such as *Acidovorax avenae subsp. citrulli*, *Agrobacterium tumefaciens*, *Clavibacter michiganensis subsp. michiganensis*, *Erwinia amylovora*, *Pseudomonas*



syringae pv. *tomato*, *Ralstonia solanacearum* and *Xanthomonas campestris* pv. *vesicatoria* were distinguished by measuring the VOCs produced from pure cultures using e-nose and discriminant function analysis (Momol et al. 2004). Its application has also been utilized in the detection of bacterial attack in cottonwood, detection and identification of fungal forest pathogens like *Ceratocystis fagacearum* and other wood rotting fungi (Wilson et al. 2005). It has also been used in paper samples in library, archives and museum for detection of moulds like *Aspergillus* or *Eurotium species* growing on them (Canhoto et al. 2004). Electronic nose system-based technique was evaluated to detect fire blight in pear and give an early diagnosis. Abbe Fetel cultivar of pear plants were maintained under controlled environmental conditions and inoculated with *Erwinia amylovora*. Metal-oxides semiconductor-based sensor array was used to analyze the VOCs synthesized by plants. These were constructed out of a heated ceramic core coated by a semi-conducting film. The semiconductor sensors could detect volatiles by monitoring changes in the conductance during oxidative reaction of molecules present in the gas phase (Spinelli et al. 2004).

Balasubramanian et al. (2007) evaluated an artificial olfactory system for determination of grain quality. Under damp storage conditions fungi like *Penicillium*, *Aspergillus* and *Fusarium* grow and synthesize mycotoxins. The growth of mould decreased the quality as well as germination of grains resulting in economic loss. *Penicillium* and *Aspergillus* infected seeds under storage conditions whereas *Fusarium* caused damage under the field conditions by producing a toxin called deoxynivalenol. Electronic nose biosensors precisely and quickly identified the metabolic activity of fungus in stored grains by analyzing the VOCs produced. The gas chromatography analysis revealed that the compounds like 2-methyl-1-propanol, 3-methyl-1-butanol, 1-octen-3-ol, and 2-methoxy-4-ethyl-phenol were synthesized by the fungi. Presence of compounds belonging to functional groups like alcohol, aldehyde, ester, ketone, acetate, and furans were detected from the headspace of the barley grain samples.

Laothawornkitkul et al. (2008) worked on plant volatile signatures and e-nose was used to make the distinction between the VOCs released by tomato, pepper, and cucumber leaves that had been harmed by pests and diseases and unharmed control leaves. The data was assessed using principal component analysis, discriminant function analysis and cluster analysis. The findings showed that it could distinguish between leaves of the same plant species that were naturally injured and those that were purposefully damaged. In cucumbers, the e-nose could distinguish between volatile organic compounds released from control, artificially damaged, and spider mite-infested leaves. Additionally, it could distinguish between VOCs released from tomato leaves that were infected with powdery mildew, intentionally injured, hornworm-damaged, and control. Gas chromatography mass spectrometry was used to quantify the correlations between the changes in volatile signatures identified by the e-nose and the changes in the underlying chemistry of plant VOC



signatures in response to applied stressors. E-nose technology can hence be applied in agricultural and horticultural contexts as a real-time pest and disease monitoring system.

Markom et al. (2009) used an E-nose system to detect basal stem rot disease caused by *Ganoderma boninense* fungus in oil palm plantation during field experiments. They used a commercially available electronic nose, Cyranose 320 as frontend sensors and artificial neural networks for pattern recognition and obtained results which showed that the system was able to differentiate healthy and infected oil palm tree using different odor parameters with a high rate of accuracy.

Pan et al. (2014) investigated the performance of E-nose in detection and discrimination of strawberry fruits infected by three post-harvest pathogens i.e., *Botrytis* sp., *Penicillium* sp. and *Rhizopus* sp. In the early storage stage. The differences in VOCs observed in infected fruits and control were further analyzed by HS-SPME-GC-MS to evaluate the changes in profile of VOCs produced by the fungal infection. The discrimination accuracy of the fungal infection in strawberry fruits reached 96.6% by using multilayer perceptron neural network model. It gave an early diagnosis and distinctly classified the pathogens. It proved that e-nose biosensor can be successfully utilized as an effective tool to reduce post-harvest losses caused by fungi during storage (Pan et al. 2014). Similarly, successful applications of E-nose have been reported to detect fungal infections in peaches, rice kernels, golden delicious apples, pomegranate and garlic. Recently, Xu et al. (2023) developed E-nose for detection of Huanglongbing (HLB) disease of citrus caused by phloem colonizing bacterium called *Candidatus liberibacter asiaticus* and detected 175 VOCs in three categories of plant samples viz., healthy, HLB and zinc deficiency. They used the headspace solid-phase microextraction gas chromatography-mass spectrometry (HS-SPME-GC/MS) which presented the differences in VOCs available in different sample categories. They set up multiple sets of comparison experiments and observed that the best collection was achieved when sample of 0.2g was collected within 20 minutes at 40°C temperature with 200ml of headspace volume. Healthy, HLB-positive, Zn-deficiency and HLB-positive were the four types of samples used for mode reliability validation, which gave an accuracy of 97.79% for HLB samples for multiple symptoms (including HLB-positive and Zn-deficiency and HLB-positive) identification. It showed an accuracy of 96.43% in samples with a combined effect of Zn-deficiency and HLB. This study showed that E-nose based method of HLB detection in citrus is efficient in suppressing the spread of HLB, which ensures good quality of citrus and decreases economic loss.

Advantages of E-nose biosensors

1. **Sensitive:** These are highly sensitive, specific devices and can detect very low concentrations of target analytes.



2. **Early Detection:** E-nose biosensors can detect plant diseases at an early stage, often before visible symptoms appear. This early detection allows for timely intervention and management, preventing the spread of diseases and minimizing crop losses.
3. **Rapid:** Provide results quicker as compared to traditional methods.
4. **Portable:** Can be designed as portable devices for on-site testing in field allowing easy use even in field and nursery conditions.
5. **Multiplexed:** these have the potential to detect multiple analytes from a mixture simultaneously.
6. Calorimetric or catalytic bead E-nose biosensors give a fast response and is highly specific for oxidized compounds.
7. Catalytic field-effect sensors are small in size therefore, portable and with a low operational cost.
8. Conducting polymer sensors is sensitive to multiple VOCs with a quicker response time, diverse sensor coatings and resistant to sensor poisoning.
9. Electrochemical sensors consume less power and is also sensitive to diverse VOCs.
10. Metal oxides semi-conducting sensors are highly sensitive with limited sensing range, rapid response and recovery times for low molecular weight compounds.
11. Optical sensors show high sensitivity and identify individual compounds in mixture with multi-parameter detection capabilities.
12. Quartz crystal microbalance (QMB) sensors have high precision with diverse range of sensor coatings and high sensitivity.
13. Surface acoustic wave (SAW) sensors have high sensitivity, diverse sensor coatings, inexpensive and sensitive to mostly all gases.

Disadvantages

1. **Specificity:** it is crucial to ensure that the biosensor component is highly specific to the particular target analyte.
2. **Stability:** biological sensing element should have the capacity to remain stable over time irrespective of different conditions.
3. **Cost:** its development and maintenance cost can be expensive.
4. Catalytic field-effect sensors require environmental control and baseline drift has low sensitivity to ammonia and carbon dioxide.
5. Conducting polymer type e-nose sensors are sensitive to humidity and temperature.
6. Electrochemical sensors are bulky in size with limited sensitivity to low molecular weight gases.



7. Metal oxides semi-conducting sensors require high power consumption, have limited sensor coatings and are sensitive to humidity.
8. Optical sensors have complex and expensive sensor-array systems with low portability due to delicate optics and electrical components.
9. Quartz crystal microbalance e-nose biosensors have poor signal to noise ratio and are affected by change in humidity and temperature.
10. Surface acoustic wave e-nose have a complex circuitry, are temperature sensitive and the specificity to analyte groups is affected by polymeric- film sensor coating.

Conclusions

E-noses are sensing devices that consist of a sensor array capable of producing a digital fingerprint of VOCs released from any source. The profile of volatile metabolites released by plants can be utilized as a disease monitoring tool for early and rapid detection of plant diseases. E-nose biosensors can be designed for field applications, allowing for real-time monitoring of plant health in agricultural settings. This capability is crucial for implementing prompt disease control measures. Integrating the e-nose biosensor into practical agricultural settings, ensuring ease of use and interpretation of results by farmers or agricultural professionals is needed. Development of novel sensor materials, improved data analysis algorithms for pattern recognition and conducting field trials to validate performance under real-world conditions is required. In conclusion, e-nose biosensors offer a promising technology for early and non-destructive detection of plant diseases by detecting unique VOCs profiles emitted by infected plants. Their application in agriculture holds potential for improving disease management strategies and enhancing crop yield and quality.

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