Methodology for the bacteria detection in drinking water through an e-nose and e-tongue

Metodología para la detección de bacterias en el agua potable a través de una nariz electrónica y lengua electrónica

DOI: http://doi.org/10.17981/ingecuc.17.1.2021.13

Artículo de Investigación Científica. Fecha de Recepción: 14/11/2019. Fecha de Aceptación: 22/10/2020.

Jeniffer Katerine Carrillo Gómez
Universidad de Pamplona. Pamplona (Colombia)
jeniffer.carrillo@unipamplona.edu.co

Cristhian Manuel Durán Acevedo
Universidad de Pamplona. Pamplona (Colombia)
cristhianmanuel@unipamplona.edu.co

Ramón Ovidio García-Rico
Universidad de Pamplona. Pamplona (Colombia)
ovidio.garcia@unipamplona.edu.co

To cite this paper:
J. Carrillo Gómez, C. Durán Acevedo & R. García Rico, "Methodology for the bacteria detection in drinking water through an e-nose and e-tongue", *INGECUC*, vol. 17. no. 1, pp. 165–173. DOI: http://doi.org/10.17981/ingecuc.17.1.2021.13

Abstract

Introduction— The evaluation of water quality remains a challenge for public health institutions today. One of the most abundant bacteria and the one that is mainly related with the sanitary risk of water is Escherichia coli (E. coli). The incidence of this bacteria shows that there is an increased risk of the presence of other bacteria and viruses of fecal origin, many of which are pathogenic. Nowadays, standardized and regulated conventional techniques are used for the detection of E. coli in water. These techniques require at least 24-28 hours of incubation for detection; in addition to require reagents and qualified personnel, among other requirements.

Objective— This article presents an analysis of the ability of the sensory perception systems (e-nose and e-tongue) to determine and discriminate E. coli from other related bacteria in water samples.

Methodology— To verify discrimination between bacteria, water samples contaminated with three bacteria were prepared: E. coli, Klebsiella oxytoca and Pseudomonas aeruginosa. Sterilized drinking water was used as a negative control. On the other hand, to evaluate the potential of the systems under study for the detection of E. coli in drinking water, water samples from the drinking water treatment plant (DWTP) of the municipality of Toledo (N. S) were analyzed. For this, the microbiological membrane filtration method was used as reference in this study.

Results— The water samples discrimination was carried out through the Principal Components Analysis (PCA), reaching 97.6% of variance captured through the electronic nose. On the other hand, with the electronic tongue, the discrimination of the bacteria was 99.4% variation in the data set, obtaining a similar response with both methods.

Conclusions— The results confirmed that the methodology allowed an effective evaluation between the contaminated samples and control samples. It is obtained a good discrimination of the categories for the samples acquired from the water treatment plant.

Keywords— E. coli; drinking water; electronic nose; electronic tongue; data processing

Resumen

Introducción— La evaluación de la calidad del agua sigue siendo un desafío para las instituciones de salud pública en la actualidad. Una de las bacterias más abundantes y que se asocia principalmente con el riesgo sanitario del agua es Escherichia coli (E. coli). La incidencia de esta bacteria indica que existe un mayor riesgo de la presencia de otras bacterias y virus de origen fecal, muchos de los cuales son patógenos. Hoy en día, para la detección de E. coli en agua, se utilizan técnicas convencionales estandarizadas y reguladas. Estas técnicas requieren al menos 24-28 horas de incubación para la detección; además de requerir reactivos y personal calificado, entre otros requerimientos.

Objetivo— Este artículo presenta un análisis de la capacidad de los sistemas de percepción sensorial (e-nose y e-tongue) para determinar y discriminar E. coli de otras bacterias relacionada en muestras de agua.

Metodología— Para verificar la discriminación entre bacterias, se prepararon muestras de agua contaminadas con tres bacterias: E. coli, Klebsiella oxytoca y Pseudomonas aeruginosa. Como control negativo se usó agua potable esterilizada. De otra parte, para evaluar el potencial de los sistemas en estudio para la detección de E. coli en agua potable, se analizaron muestras de agua procedentes de la planta de tratamiento de agua potable (DWTP) del municipio de Toledo (N. S). Para ello, el método microbiológico usado como referencia fue el de filtración por membrana.

Resultados— La discriminación de las muestras de agua se realizó mediante el Análisis de Componentes Principales (PCA), alcanzando el 97.6% de la varianza capturada a través de la nariz electrónica. Por otro lado, con la lengua electrónica, la discriminación de la bacteria fue una variación del 99.4% en el conjunto de datos, obteniendo una respuesta similar con ambos métodos.

Conclusiones— Los resultados demostraron que la metodología propuesta permitió una evaluación efectiva entre las muestras contaminadas y las muestras de control. Se observa una excelente discriminación de las categorías para las muestras obtenidas de la planta de tratamiento de agua potable.

Palabras claves— E. coli; agua potable; nariz electrónica; lengua electrónica; procesamiento de datos

© The author; licensee Universidad de la Costa - CUC.

I. Introduction

Nowadays, drinking water preparing is a procedure that includes compliance with certain physicochemical and microbiological parameters that are regulated by water quality rules. The control and monitoring of water for human consumption coming from Drinking Water Treatment Plants (DWTP) or aqueducts, is an important strategy to evaluate the operational efficiency of the plant and standardize the operational parameters to produce high-quality water [1]. Regarding, at the international level, the World Health Organization (WHO) establishes that drinking water for human consumption must be free of any microorganism that may affect the health of the consumer, this parameter is used as a quality guideline for the creation of regulations or standards. It also serves to assess contamination risks, monitoring processes and make corrective plans to achieve the desired quality. In Colombia, the microbiological quality only includes the control of bacteria such as total and fecal coliforms where the admissible values are contained on the resolution 2115/2007 of article 25, which indicates that the allowed value for these microorganisms must be zero for every 100 ml, when it comes to water for human consumption.

Additionally, in this normativity are stipulated the different conventional methods (i.e., fermentation of multiple tubes, filtration by a membrane, enzyme-substrate, defined substrate, among others) used for the detection of bacteria, however, it must be emphasized that these methods are complex, they represent high costs, extensive analysis times since they are still an obstacle to establish in a determined time the microbial quality of the water for human consumption.

Nowadays, one way to verify the microbiological quality of water is to constantly monitoring a bacterial indicator as important as *E. coli* [2]. However, there is a great limitation at the moment of analyzing this bacterium in most of the drinking water treatment plants, since they do not have worthy laboratories, specialized technologies, and trained personnel that allow performing these microbiological tests, limiting the frequency of the analysis (i.e., 1 to 2 times a month). Likewise, samples must be sent to certified laboratories generating costs, errors derived from sampling that can alter the analyzes, and delays in obtaining results. It is for this reason that the limitations of conventional methods have led to research focusing on the development of fast and accurate methods for bacteria identification.

For real-time monitoring, many investigations have focused on the development of fast and accurate methods for their detection, such as the use of electronic nose and tongue technologies used for the specific analysis, identification, and recognition of volatile organic compounds emanated by the bacterial species. At present, several studies have been done using the nose and electronic tongue [3]-[12]. In this study, it was proved that both the e-nose and e-tongue are techniques that allow obtaining results in a fast way for the discrimination and identification of the bacteria.

II. Background

Traditionally the methods used for the microbiological analysis of foods are based on the ability of the microorganisms to grow under artificial conditions. Culture-based methods have been the classical methodology for bacterial detection, even the pathogenic strains. These methods give a confirmed result regarding the presence of a particular pathogen and the success rate is found to be high. These methods are sensitive, reliable, low-cost, and provide both qualitative and quantitative results on the bacterial populations present in the sample [13]. However, the biggest drawback in the culture-based method is the slow growth due to which excess time is lapsed to get the final result, which can turn out to be fatal. It must be noted that all these media take up to 18-24 h to give the exact result, indicating the slow turnaround time. Even the isolation of pure cultures applying several growth steps, is necessary to characterize the bacteria in more detail. Consequently, the conventional methods are labor-intensive and time-consuming, since the results are not available until at least 1-3 days [14], [15]. One of the best-known examples is the culture of E. coli O157: H7 on Sorbitol, which is based on the principle of fermentation of sorbitol. However, the major limitation in this method is slow turnaround time and false-positive results due to the emerging sero-

types of sorbitol fermenting E. coli non-O157 [16]. On the other hand, many microorganisms tend to enter the starvation mode of metabolism under stress conditions. However, they will remain Viable But Not Cultivable (VBNC), so they will not develop in conventional culture media, but may indicate virulent pathways [17]. That is, culture-based methods reach their limits with specific microorganisms that do not grow on or in artificial media. The detection of these pathogens is a major challenge for food safety. Nowadays, most water samples that are analyzed by instrumental techniques are sometimes complex or incompatible with the Gas chromatography and Mass Spectrometry (GC-MS) analytical equipment, which prevents the automatic supply of samples towards the measurement system. The above requires a prior treatment of the sample, before performing the analysis [18]. Chromatography is an analytical method widely used in different applications due to its great power to analyze samples with a variety of compounds. The main operation of this type of equipment is based on the retention (differential) of analytes by the stationary phase and then they are transported by a mobile phase along a column. Chromatographic techniques can be established based on the function of the mobile phase: Liquid Chromatography (HPLC) and Gas Chromatography (GC). On the other hand, Mass Spectrometry (MS) is considered a useful tool for bacterial identification [19] owing to the sample classification, its rapidness, and high dynamic range. However, as was mentioned above these system requires expert personal, expensive, and take a long time to obtain results.

During the last decade, research has been carried out that seeks to take advantage of the advantages of electronic smell (E-nose) and electronic tongue (E-Tongue) systems as a tool for evaluating the quality control of products in the environmental, agro-industrial sector, health, environment, among others. An electronic nose consists of an equipment combined with a matrix of chemical gas sensors, a stage of sample conditioning, a data acquisition system with associated electronics, and different algorithms for pre-processing and data processing applied to obtain a relevant response on the compound to be identified [20].

In the present study, an E-nose was used to detect the odor generated by bacteria since they generate a range of volatile organic compounds (VOCs) that develop as products or by-products of metabolic pathways; for example, the generation of hydrocarbons, aliphatic alcohols, and ketones from the biosynthesis of fatty acids, the evolution of indole (responsible for the putrid odor associated with E. coli) from the breakdown of the amino acid tryptophan [21].

Furthermore, an electronic tongue is composed of a potentiostat with several channels which are generally configured employing the Cyclic Voltammetry (CV) method which is applied to a data set of liquid samples (i.e., water, milk) This electrochemical technique can measure the current that changes in an electrochemical cell under conditions where the voltage supplied depends on the electrode capacity. The electrodes are performed of different materials such as Gold, carbon, nanotubes, graphene, platinum, and others [22]. When the Working Electrodes (WE) are in contact with the liquid samples, the electrical current varies depending on the analyzed substance. At the end of the reduction/oxidation process, a cyclic voltagram is obtained by measuring the current at the WE during the potential scanning.

Numerous data pre-processing and processing techniques are used for data analysis, where the information can be used through pattern recognition algorithms. There are several types of scaling techniques such as linear, logarithmic, variables, or measures that allow improving the system response. In this study, four standardization methods were used: Auto-scaling, mean centering, column normalization, and matrix, and all measurements were analyzed by using a multivariate statistic technique called Principal Component Analysis (PCA). This multivariate analysis method is widely useful in feature reduction, data compression, variable selection, and sometimes used for noise reduction. This statistical powerful procedure for data analysis was selected in this application since it is an effective linear unsupervised method to extract the most relevant information and project data from several sensors to a two-dimensional plane using scores plot. Therefore, it is possible to discriminate properly a data set in a non-supervised way, finding the directions of maximal variance. PCA returned a new basis which is a linear combination of the original basis [23].

III. Methodology

A. *Bacterial strains*

In this study, two bacteria of the Enterobacteriaceae family were used: Escherichia coli (ATCC 25922) and Klebsiella oxytoca (ATCC 49131) as representatives of the group fecal coliforms and total coliforms, respectively. Additionally, Pseudomonas aeruginosa (ATCC 27853) was used since it is a Gram-negative bacillus of importance in drinking water, which does not belong to the Enterobacteriaceae. The strains were conserved and supplied by the microbial collection center of the University of Pamplona.

B. *Samples preparation*

Each of the bacteria was grown in nutritive agar at 37°C/24h. From isolated colonies, 10 ml of a bacterial suspension was prepared in vials with sterile water and with an approximate concentration of 3×10^8 CFU/ml by using the standard No. 1.0 of the McFarland scale. Afterward, this solution was diluted 300 times (1/300) to a final volume of 300 ml of sterile water, obtaining a final concentration of 1×10^6 CFU/ml. Before analysis, each sample was incubated for 12 hours at 37°C to improve the enrichment of the compounds generated. For each strain, 20 ml were taken in a vial to be analyzed by the e-nose and e-tongue and the analysis was repeated ten times. This procedure was performed to estimate the ability to discriminate between related bacteria. Besides, sterilized drinking water at 121°C/15 psi/15 min was used as a negative control. The number of measurements acquired with the e-nose system was 40 samples and with the e-tongue system were acquired 42 samples.

C. *Drinking water sampling*

To make a preliminary analysis of the ability of these sensory perception systems to detect *E. coli* in water samples during water treatment a conventional microbiological method was applied from a Drinking Water Treatment Plant (DWTP). For this, the DWTP of the municipality of Toledo located in North of Santander (Colombia) was used. The sampling points were the following: the entrance of the plant (input water), after the sedimentation (sediment-water), the outlet from the plant (output water), and water from the tap of a house (tap water). A contaminated water sample with *E. coli* was prepared as described above, and sterilized drinking water was used as a negative control. On the other hand, the membrane filtration method was the microbiological methodology used in this study. The filtration was performed as follows: a sample of 100 ml of water was passed through a 47-mm diameter cellulose sterile filter (Millipore 0.45-µm) and transferred to a plate on Chromocult® Coliform Agar (Merck) to allow the growth of bacteria for 24 h at 35 ± 2°C.

Sterile water samples and contaminated samples with *E. coli* were prepared in the microbiology laboratory at a concentration of 1×10^7 CFU/ml which were taken as a reference to determine in which place or quadrant of PCA plot are projected the measurements of the plant. The sensitivity of the methodology was calculated from the sensors responses numerically and objectively. To determine the sensitivities of each sensor, a parameter selection method widely used in metal oxide sensors was applied. In this case, it was considered that the most appropriate would be the increase in the normalized conductance X given by (1).

$$X_{ij} = \frac{G_{max} - G_i}{G_i} \tag{1}$$

Where, is the maximum value of the signal in conductance value or the highest intensity of the presence of the bacteria, and is the initial value of the signal's conductance. Therefore, the increase of the amplitude of the signal indicates the sensitivity degree of the system from gas sensors. Likewise, electronic tongue sensitivity was determined similarly through (2), wherein this case indicates the maximum response of the electrode at the moment of detecting the liquid sample (oxidation) and is the current response at the time of deoxidation of the sample.

$$M_{ij} = I_{max} - I_{min} \quad (2)$$

To determine the selectivity of both systems, the PCA plot was used to verify the behavior between the different clusters of bacteria and the sterile water of both systems. In this case, the behavior of the sensory systems was assessed by evaluating the ability to distinguish one category from another.

To have a volume of 300 ml of sample, the bacterial suspensions were diluted 30 times in sterile water and incubated for 12 hours at 37°C, to improve the enrichment of the compounds generated.

D. *Electronic Nose and Electronic Tongue*

Fig. 1a illustrates each of the stages that were carried out for the development of the perception systems to make the experimental tests. The procedure was carried out by an electronic nose performed in the electronics laboratory at the University of Pamplona (Colombia) for the detection of Volatile Organic Compounds (VOCs) emitted by bacterial species in drinking water. To increase the concentration of the analytes, the samples were conditioned at 50°C through a static headspace system for 10 minutes. Once the incubation time and temperature were reached, the samples were extracted through switching an electric-pump and then transferred to a measuring chamber (methacrylate material) equipped with a matrix of 16 commercial sensors of metal oxides with overlapping's sensitivities. The operation of the static headspace system was based on the temperature control of a heating resistor and the operation of a stepper motor for automatic control of the injector. The temperature of the heating capsule was regulated by a Proportional, Integral and Derivative (PID) controller of Discrete-Type Implemented in a low-cost data acquisition card (Arduino Mega, 2560). A 20 mL vial was sealed by a septum that was placed inside the capsule to apply and maintain the temperature at 50°C and generate the volatile components to a steam phase (upper space of the road known as "Headspace"). Through a user graphical interface, the incubation time was also necessary to generate the compounds and the temperature of the sample in a range of 0°C to 110°C was fixed. For data acquisition and collect samples, a USB-6211 board was used from the sensor chamber to store all the information to a computer. Besides, Fig. 1b shows a flow diagram of the different stages developed to make the tests by using the e-nose and e-tongue systems.

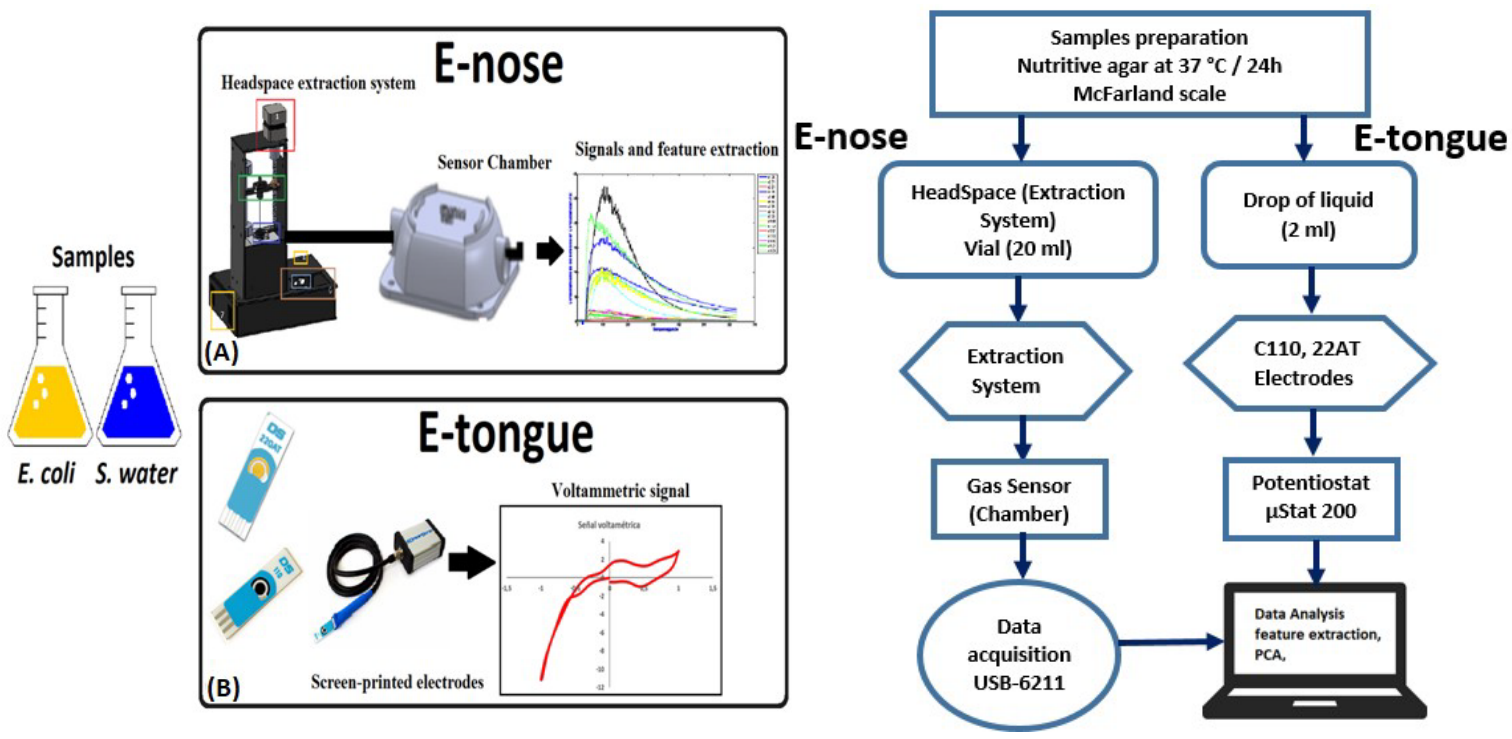

Fig. 1. (a) General scheme of the methodology implemented ((A) E-nose and (B) E-tongue), (b) Flow diagram of both systems.
Source: Authors.

Table 1 describes the main characteristics of the commercial gas sensors to perform the electronic nose system [14].

Table 1. Commercial gas sensor.

Variable (V)	Sensor	Target Gas
1	TGS 826	Ammoniate and amine
2	TGS 831	R-22 Monoclorodifluoromethane
3	TGS 821	Hydrogen
4	TGS 826	Ammoniate and amines
5	TGS 842	Methane and natural gas
6	TGS 880	Smoke of the food (Alcohol, odour)
7	TGS 825	Hydrogen sulphide
8	TGS 813	Hydrocarbons in general
9	TGS 800	Air pollutants in general
10	TGS 880	Smoke of the food (Alcohol, odour)
11	TG0S 822	Alcohol and organic solvents
12	TGS 821	Hydrogen
13	TGS 832	R-134[a] 1,1,1,2-Tetrafluoroethane
14	TGS 842	Methane and natural gas
15	TGS 831	R-22 Monoclorodifluoromethane
16	TGS 830	R-22 Monoclorodifluoromethane

Source: Authors.

Fig. 1b illustrates the different stages to perform the electronic tongue, which consists of a portable potentiostat (1) µSTAT200 manufactured by Dropsens company with carbon-printed electrodes: C110 and gold electrodes: 220AT.

The DropView 200 software was used to configure the potentiostat, where the cyclic voltammetry technique was applied with the following parameters: Ebegin: 0, indicates the potential where the scanning starts, Evtx1: -2 (Volts) is the potential where reverses the scanning direction, Evtx2: +1 (Volts) is the potential where the scan direction is reversed again or where scanning stops (yes Evtx2 = Ebegin), Srate (V / s): 0.5, scan time and nscans: 1, number of scans.

The current range was placed in automatic mode. It is important to note that a higher current is selected when the current exceeds 2 times the applied current. A lower current is selected when the measured current is less than 0.05 times the applied current range. The selectable current ranges are specified in the "Method" tab. A drop of liquid of 2 ml of each sample was applied to the C110 and gold electrodes.

Table 2 presents the characteristics of the sensors used in the different tests.

Table 2. Screen-Printed Electrodes

Sensor	DRP-220AT (Gold)	DRP-C110 (Carbon)
Electrical Contact	Silver	Silver
Working Electrode	Gold	Carbon
Counter Electrode	Gold	Carbon
Reference Electrode	Silver	Silver

Source: Authors.

E. *Data processing*

The pre-processing and data processing were carried out by methods of extraction of parameters to obtain the maximum information from the data set. Likewise, standardization and pattern recognition techniques were used, where the static parameter (V_{max} and V_{min}) was initially obtained, corresponding to the maximum voltage and the minimum voltage reached by each of the gas sensors. Afterward, the data were normalized using the column normalization technique with regards to the e-nose and self-scaling for the e-tongue. The data processing was done with the PCA analysis method (discriminatory method). It is worth mentioning that data processing methods were used for two sensory perception systems. Moreover, the variables used in this study were 16 units and the loadings plot was made to compare the scores plot with the discrimination of the measures.

IV. Results

Fig. 2 shows good discrimination of the categories by using PCA, where PC1 and PC2 obtained the maximum variation of the data. The percentage reached was 97.9% of the variance obtained with the first PCs, indicating that the electronic nose is capable of differentiating *E. coli* bacteria from other bacteria that are similar and may tend to confusion as is the case of *Klebsiella oxytoca* and *Pseudomonas aeruginosa*.

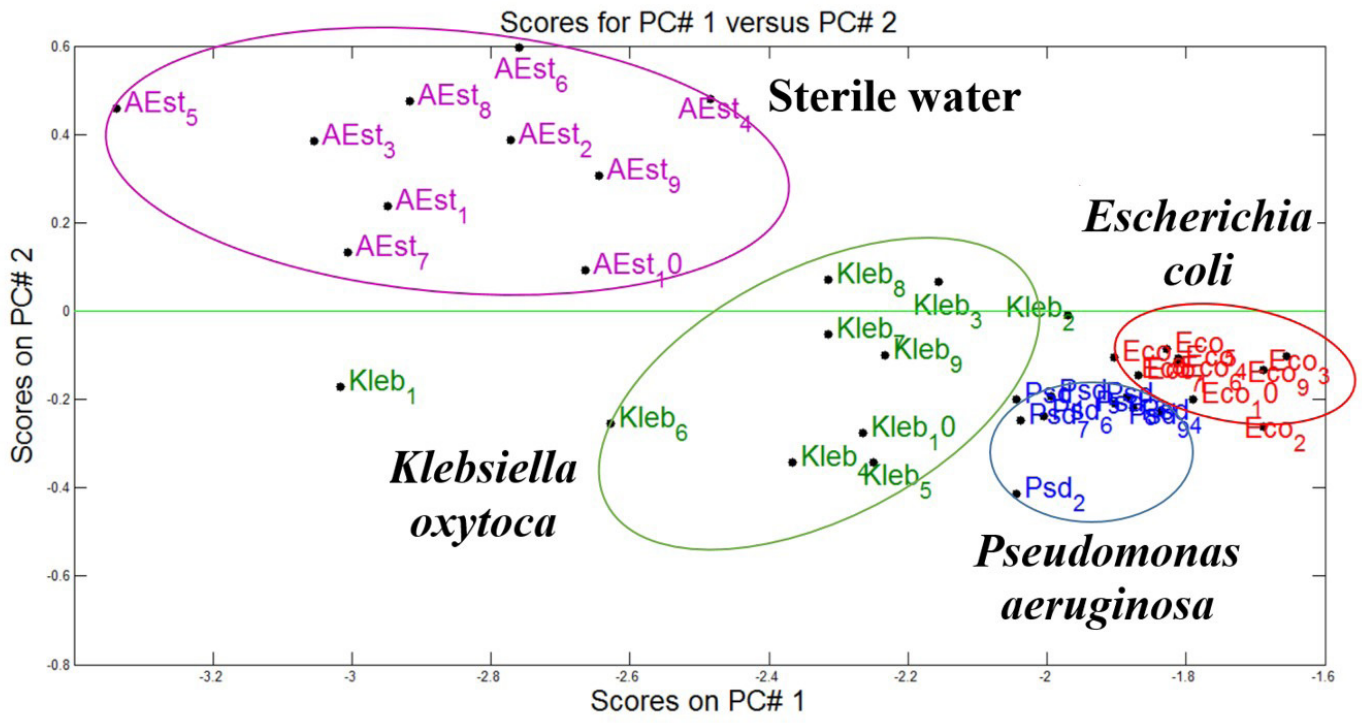

Fig. 2. PCA analysis of bacteria using electronic nose.
Source: Authors.

The e-nose achieved very good discrimination between the bacteria tested, despite being associated with bacteria. It should be taken into account that the three strains tested correspond to *Gram-negative*, *K. oxytoca and E. coli* that belong to the same bacterial family (they are *Enterobacteriaceae*). The e-nose was also able to discriminate between the three bacteria and separate them from the negative control (sterile water).

As mentioned above, Fig. 3 shows the loadings plot obtained with the PCA analysis, where the projection of the original variables indicates the contribution of each of them to the discrimination of the categories. In Fig. 2 and Fig. 3 the influence of each of the variables related to each of the principal component axes (PC1 and PC2) contains the maximum variation of the data set.

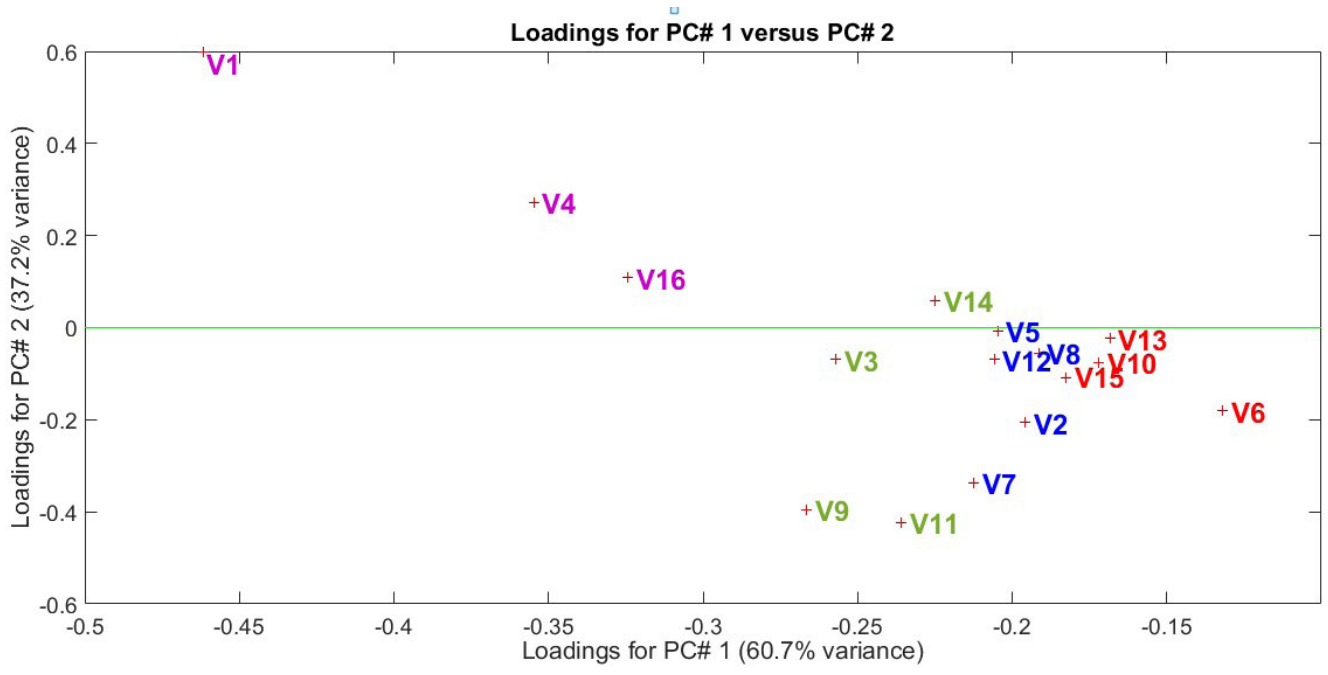

Fig. 3. Loadings plot of bacteria using E-nose.
Source: Authors.

Regarding Table 1 and Fig. 3, the variables or sensors such as TGS826 (V1), TGS826 (V4), and TGS830 (16) were more sensitive to the halocarbons that it is related to the sterile water. Besides, TGS821 (V3), TGS800 (V9), TGS842 (V14) and TGS822 (V11) sensors responded to as odor detection that were related to *Klebsiella oxytoca*. On the other hand, TGS831 (V2), TGS842 (V5), TGS825 (V7), TGS813 (V8) and TGS821 (V12) sensors which were more sensitive to alcohols that were related to *Pseudomonas aeruginosa*, and finally the TGS880 (V6), TGS880 (V10), TGS 832 (V13) and TGS831 (V15) sensors were linked to *E. coli* bacteria as they responded to alkenes and ketones.

It was considered the case where all the samples of the three bacterial species and sterile water were combined in a single data set that was heated to 50°C to generate a reliable headspace for the VOCs extraction. Fig. 4 presents the results obtained with the electronic tongue in which Fig. 4a shows excellent discrimination of the categories with a variance percentage of 99.4%, using the gold sensor. On the other hand, in Fig. 4b the result of the carbon sensor was obtained, achieving a total variance of 96.27%. Besides, the e-tongue (C110 electrode) efficiently discriminates the *E. coli* and achieves a good separation from the negative control, but it does not do so well with the other two bacteria, as the sterile water samples are projected with a certain closeness to *K. oxytoca and P. aeruginosa* samples (22AT electrode) (Fig. 4).

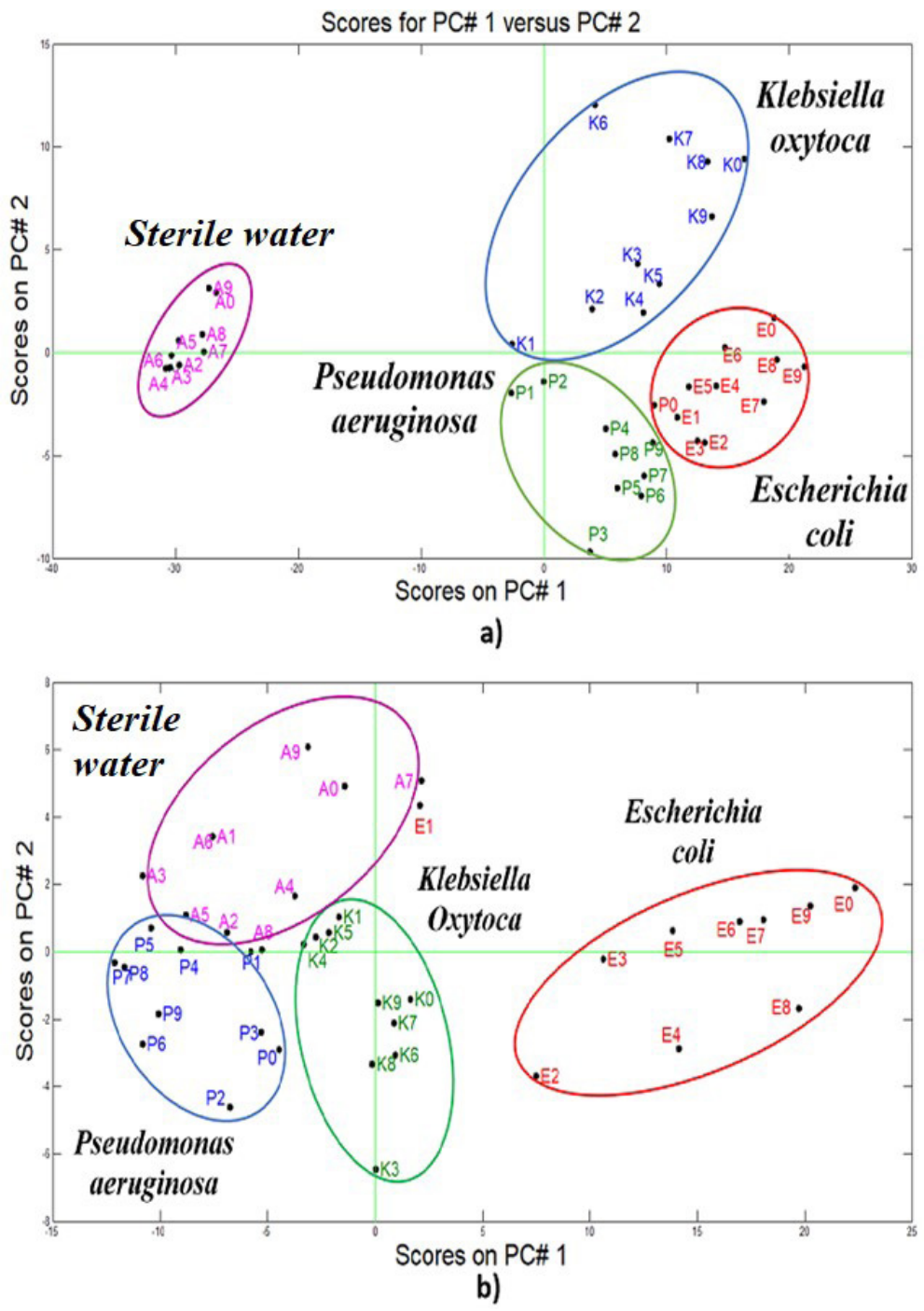

Fig. 4. PCA Plot: a) 22AT electrode, b) C110 electrode.
Source: Authors.

With these preliminary results of discrimination of the three bacteria, the analysis was carried out with the samples acquired from the Toledo plant located in Norte de Santander (Colombia). Fig. 5 and Fig. 6 show the discriminations of the samples collected at the four collection points (i.e., Output-water, Tap-water, sediment-water, and input-water of Toledo's aqueduct).

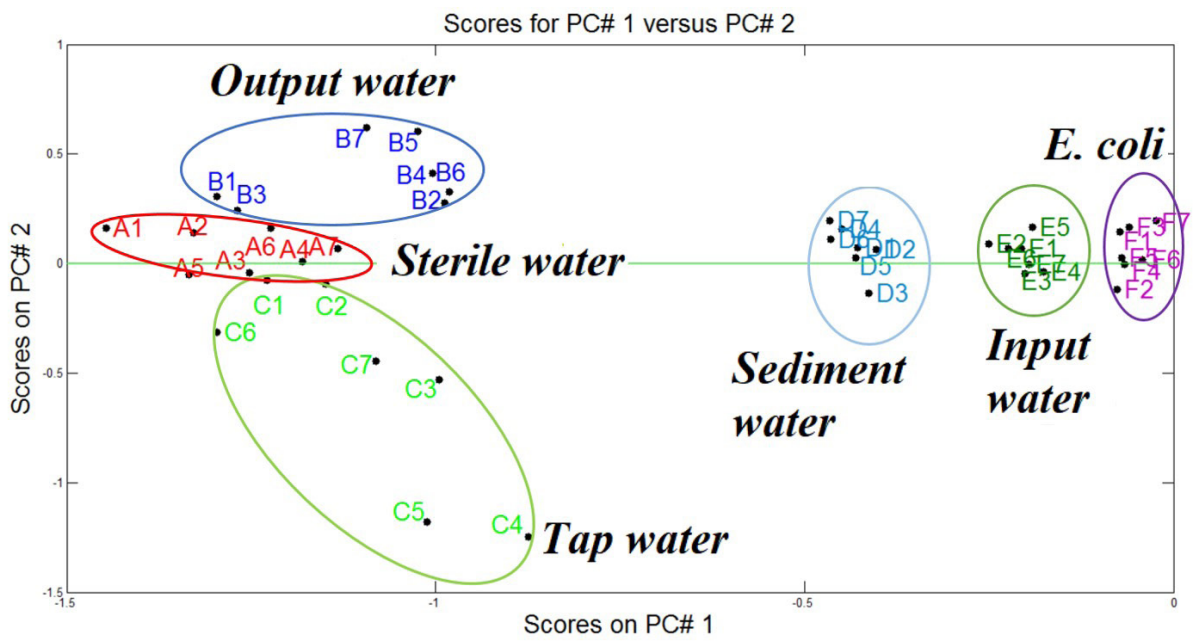

Fig. 5. PCA plot. Samples discrimination from Toledo's plant by using the electronic nose system.
Source: Authors.

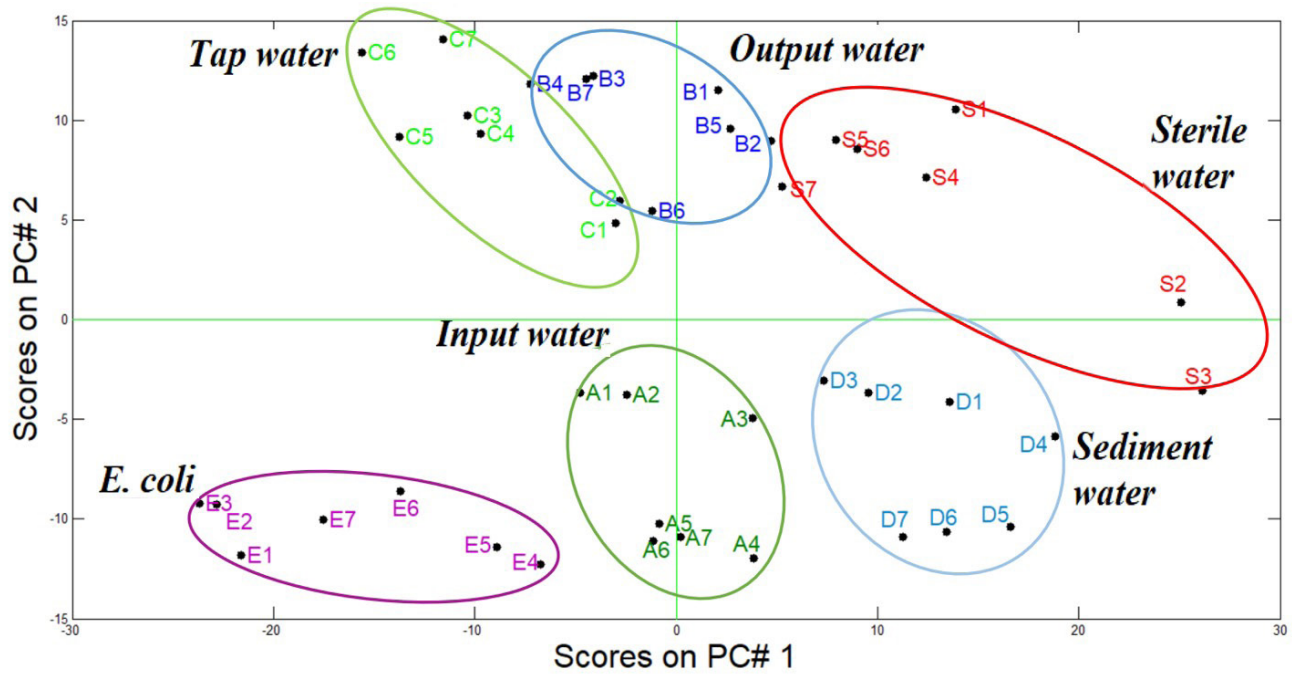

Fig. 6. PCA plot. Samples discrimination from Toledo's plant by using the electronic tongue system.
Source: Authors.

One of the most important characteristics that can be observed in Fig. 5 is the difference between the categories by using the first two scores of the PCA, obtaining 98.5% of the variance in the case of the electronic nose the gas sensors detect "*all or nothing*" volatile compound pattern that identifies the characteristic of the smell of each sample. They do not detect compounds in a specific way, but rather a trace equivalent to that pattern.

It is very important to clarify that the microbiological analysis was possible to identify the compounds present in *E. coli* bacteria by using a database called "mVOC" [24]. This database describes several microbial volatile compounds about *E. coli* bacteria. The following information shows the different compounds emitted by *E. coli* bacteria: On the information is describes the *Name, Formula, and chemical classification* of each compound such as: Decan-1-ol C10H22O (Alcohol), Dodecan-1-ol CH3(CH2)10CH2OH (Alcohol), Nonan-2-one C9H18O (Ketone), Octan-1-ol C8H18O (Alcohol), Tridecan-2-one C13H26O (Ketone), Undec-1-ene C11H22 (Alkenes) and Undecan-2-one C11H22O (Ketone). Thus, the sensors responded very well to the contaminated and non-contaminated water samples.

Fig. 6 depicts the results employing the C110 electrode that was also used to determine the reliability of the electronic tongue in the detection of *E. coli* bacteria, thus verifying the correct functioning of the sensory system. The PCA analysis also confirms the use of the e-tongue for the discrimination of water samples in the different collection points, obtaining a variance percentage of 86.3% with the first two PCs.

To validate the results obtained from Fig. 4 and Fig. 5, the membrane filtration method was used in a parallel way. Following the microbiological requirements required by Colombian resolution 2115, those samples that exceed the value of the indicator taken as the limit of acceptability (0 ml CFU/100 ml of water) are considered as samples contaminated with *E. coli*.

From the results obtained with the nose and the electronic tongue, it can be deduced that in the treatment plant the samples of exit water and tap water are grouped in the category of free samples of *E. coli* bacteria, is located near the sterile water reference samples projected on the PCA chart. As was mentioned above, this result coincides with the concentration obtained by the membrane filtration method which was 0 CFU/ml in both cases. Table 3 illustrates the same behavior, it can be observed with the samples contaminated by *E. coli*, but analyzing by the membrane filtration method.

TABLE 3. MICROBIOLOGICAL ANALYSIS USING THE METHOD OF MEMBRANE FILTRATION.

Target	Input water	Sedimentation water	Output water	Tap water
E. coli	125 UFC/ml	26 UFC/ml	0 UFC/ml	0 UFC/ml

Source: Authors.

The e-nose grouped two samples that according to the microbiological analysis were negative for *E. coli* and both with the negative control (sterile water). Additionally, these three sets (output water, tap water, and sterile water) were well discriminated from the bacteria samples (Fig. 5). The microbiological analysis showed that the samples called input-water and sediment-water contained the bacteria (Table 3). The PCA generated a grouping pattern of the samples that ordered them according to the amount of *E. coli* they had, in increasing order from left to right. The sediment-water is the group closest to the negative samples and corresponds to the sample with the least amount of *E. coli* (26 CFU / ml), while the positive control (1 × 10 6 CFU/ml) is the furthest set. Besides, these three samples also present a low dispersion between the data (the points are very close to each other in each set). The above clearly shows us the enormous potential of this methodology for the *E. coli* detection in water. Moreover, when the e-tongue was tested with the same samples the result was not so good. The C110 electrode allowed us to obtain good discrimination between the samples, generating six sets with an excellent separation; however, a clustering pattern was not as clear and promising as that obtained with the e-nose system (Fig. 6). Apparently, for the detection and possible quantification of the bacteria in water, the volatile metabolites are more useful and informative than the non-volatile ones.

From a microbiological point of view, in the treatment plants, the population of *E. coli* presented a similar behavior. The water enters with an amount of *E. coli* above 100 CFU / ml, after the settler the load of the bacteria is diminished between 5 and 10 times and finally, the water at the exit of the plant does not present viable E. coli in 100 ml of water, as required by the norm, evidencing the efficiency of the treatment process of the plants. Besides, it was also observed that the distribution system is not a source of contamination by this bacterium, since the samples of the faucets in the houses presented negative results.

V. CONCLUSIONS

In this study, the specificity of the method was evaluated employing the capacity of the proposed methodology (electronic nose and electronic tongue) to discriminate the compounds of interest, in this case, the *E. coli* bacteria with other bacteria of similar conditions.

The compounds generated by each of the bacteria are differentiated through microbiological tests and what is obtained with the e-nose and e-tongue.

Although the better results were obtained with the electronic nose since it presented better selectivity in the discrimination of the samples, it is important to highlight that the electronic tongue also showed a very good response both with the use of the C110 and 22AT electrodes for the identification of bacteria.

The results of this tool are promising because, due to its portability, it is possible to take the respective corrective, preventive measures in an aqueduct and thus produce high-quality water for human consumption, complying with the requirements of the regulations.

As future work, it would be interesting to evaluate the methodology through the bacterial concentration and validate the capacity to discriminate and classify the different levels of bacteria. Likewise, different classification methods such as Support Vector Machine (SVM) and K-Nearest Neighbors (K-NN) could be applied to the data set to try to improve the performance of both systems.

References

[1] S. Rios-Tobón, R. M. Agudelo-Cadavid & L. A. Gutiérrez-Builes, "Patógenos e indicadores microbiológicos de calidad del agua para consumo humano," *Rev Fac Nac Salud Pública*, vol. 35, no. 2, pp. 236–247, 2017. https://doi.org/10.17533/udea.rfnsp.v35n2a08

[2] C. M. McEntegart, W. R. Penrose, S. Strathmann & J. R. Stettera, " Detection and discrimination of coliform bacteria with gas sensor arrays," *Sens Actuators B Chem*, vol. 2000, pp. 106s–116s, 2012. https://doi.org/10.1016/S0925-4005(00)00561-X

[3] J. L. Herrero, J. Lozano, J. P. Santos & J. I. Suárez, "On-line classification of pollutants in water using wireless portable electronic noses," *Chemosphere*, vol. 152, pp. 107–116, Jun. 2016. https://doi.org/10.1016/j.chemosphere.2016.02.106

[4] B. Lange, M. Strathmann & R. Oßmer, "Performance validation of chromogenic coliform agar for the enumeration of Escherichia coli and coliform bacteria," *Lett Appl Microbiol*, vol. 57, no. 6 , pp. 547–553, Aug. 2013. https://doi.org/10.1111/lam.12147

[5] N. Krishnamurthy, B.S. Supreetha, M. Deccaraman & N. Vijayashree, "E-Nose System to Detect E-Coli in Drinking Water of Udupi District," *Int J Eng Res*, vol. 1, no. 12, pp. 58–64, 2012. Available: http://www.ijerd.com/paper/vol1-issue12/H01125864.pdf

[6] J. L. Herrero, J. Lozano, J. P. Santos & J. I. Suarez, "On-line classification of pollutants in water using wireless portable electronic noses," *Chemosphere*, vol. 152, pp. 107–116, Jun. 2016. Available: https://ui.adsabs.harvard.edu/abs/2016Chmsp.152..107H/abstract

[7] I. Campos, M. Alcañiza, D. Aguado, R. Barat, J. Ferrer, L. Gil, M. Marrakchi, R. Martínez-Mañeza, J. Soto, J. L. Vivancos, "A voltammetric electronic tongue as tool for water quality monitoring in wastewater treatment plants," *Water Res*, vol. 46, no. 8, pp. 2605–2614, May. 2012. https://doi.org/10.1016/j.watres.2012.02.029

[8] G. Comina, M. Nissfolk & J. Solis, "Development of a Portable Water Quality Analyzer," *Sen Transducers*, vol. 119, no. 8, pp. 72–81, Aug. 2010. Available: https://www.researchgate.net/publication/316256844_Development_of_a_Portable_Water_Quality_Analyzer

[9] J. C. Rodríguez & C. M. Duran, "Sistema de olfato electrónico para la detección de compuestos volátiles," *RCTA*, vol. 2, no. 12, pp. 1692–7257, 2008. Available: http://www.unipamplona.edu.co/unipamplona/portalIG/home_40/recursos/02_v07_12/revista_12/16112011/v12_04.pdf

[10] C. Söderström, F. Winquist & C. Krantz-Rülcker, "Recognition of six microbial species with an electronic tongue," *Sensor Actuat B-Chem*, vol. 89, no. 13, pp. 248–255, Apr. 2003. https://doi.org/10.1016/S0925-4005(02)00474-4

[11] F. Winquist, J. Olsson & M. Eriksson, "Multicomponent analysis of drinking water by a voltammetric electronic tongue," *Anal Chim Acta*, vol. 683, no. 2, pp. 192–197, Jan. 2010. https://doi.org/10.1016/j.aca.2010.10.027

[12] J. K. Carrillo, C. M. Durán & R. O. García, "Bacterial discrimination of drinking water through of an electronic nose and a volatiles extraction equipment," *RCTA*, vol.1, no. 33, pp. 155–165, Sep. 2019. https://doi.org/10.24054/16927257.v33.n33.2019.3335

[13] B. Austin, "The value of cultures to modern microbiology," *Antonie Van Leeuwenhoek*, vol. 110, no. 10, pp. 1247–1256, Oct. 2017. https://doi.org/10.1007/s10482-017-0840-8

[14] A. M. Sousa & M. O. Pereira, "A prospect of current microbial diagnosis methods," *STE*, vol. 3, pp. 1429–1438, 2013. Available: http://hdl.handle.net/1822/33030

[15] A. van Belkum, G. Durand, M. Peyret, S. Chatellier, G. Zambardi, J. Schrenzel, D. Shortridge, A. Engelhardt & W. M. Dunne Jr., "Rapid clinical bacteriology and its future impact," *Ann Lab Med*, vol. 33, no. 1, pp. 14–27, Jan. 2013. https://doi.org/10.3343/alm.2013.33.1.14

[16] J. J. Hirvonen, A. Siitonen & S.-S. Kaukoranta, "Usability and Performance of CHROMagar STEC Medium in Detection of Shiga Toxin-Producing Escherichia coli Strains," *J Clin Microbiol*, vol. 50, no. 11, pp. 3586–3590, Nov. 2015. https://doi.org/10.1128/JCM.01754-12

[17] J.-S. Kim, N. Chowdhury, R. Yamasaki & T. K. Wood, "Viable but non-culturable and persistence describe the same bacterial stress state," *Environ Microbiol*, vol. 20, no. 6, pp. 2038–2048, Jun., 2018. https://doi.org/10.1111/1462-2920.14075

[18] P. Hernández, "Métodos cromatográficos aplicados al análisis de aguas de consumo y residuales," *M.S. Thesis*, dpto Cienc Anal, UNED. Madrid, ES, 2018. Available: http://e-spacio.uned.es/fez/eserv/bibliuned:master-Ciencias-CyTQ-Prohner/Rohner_Hernandez_Pablo_TFM.pdf

[19] C.-T. Chen, J.-W. Yu & Y.-P. Ho, "Identification of bacteria in juice/lettuce using magnetic nanoparticles and selected reaction monitoring mass spectrometry," *J Food Drug Anal*, vol. 27, no. 2, pp. 575–584, Apr. 2019. https://doi.org/10.1016/j.jfda.2018.09.006

[20] O. Canhoto & N. Magan, "Electronic nose technology for the detection of microbial and chemical contamination of potable water," *Sens Actuators B Chem*, vol. 106, no. 1, pp. 3–6, Apr. 29, 2005. https://doi.org/10.1016/j.snb.2004.05.029

[21] S. Schulz & J. S. Dickschat, "Bacterial volatiles: The smell of small organisms," *Nat Prod Rep*, no. 24, pp. 814–842, 2007. https://doi.org/10.1039/b507392h
[22] K. Tiwari, S. Biswas, B. Tudu, R. Bandhopadhyay & P. Pramanik, "Development of metal oxide-modified carbon paste based sensor for honey analysis using electronic tongue," *Mater Today: Proc*, vol. 4, no. 9, pp. 9500–9504, Mar., 2017. https://doi.org/10.1016/j.matpr.2017.06.212
[23] O. E. Gualdrón, C. M. Durán, J. E. Ortiz & J. A. Araque, "Implementacion de un modelo neuronal en un dispositivo hardware (FPGA) para la clasificacion de compuestos químicos en un sistema multisensorial (nariz electrónica)," *RCTA*, vol. 2, no. 24, pp. 127–133, Aug. 2004. Disponible en http://revistas.unipamplona.edu.co/ojs_viceinves/index.php/RCTA/article/view/2347
[24] M. C. Lemfack, B.-O. Gohlke, S. M. T. Toguem, S. Preissner, B. Piechulla & R. Preissner, "mVOC 2.0: a database of microbial volatiles," *Nucleic Acids Res*, no. 46, no. D1, pp. D1261–D1265, Jan. 4, 2018. https://doi.org/10.1093/nar/gkx1016

Jeniffer Katerine Carrillo Gómez received his B.S. in Chemical Engineering from the Pamplona University (Colombia), and she got her master studies in Industrial Controls at the same University. She is a professor, researcher and belongs to the Multisensor Systems and Pattern Recognition Research (GISM) Group at the Pamplona University. She has experience on industrial chemical process and has acquired a high knowledge in pattern recognition methods and artificial intelligence. Has worked in different projects related with microbiologic applications by using multisensory perception systems (electronic nose and electronic tongue systems). Recently, she won a national project about prostate cancer detection and currently she is involved in different european projects H2020 such as: bTB-Test (2018) and Vogas (2019). https://orcid.org/0000-0003-0392-8114

Cristhian Manuel Durán Acevedo received his B.S. in Electronic Engineering from the Pamplona University (Colombia), and he got the certificate of advanced studies and Ph.D. from the Universitat Rovira I Virgili (Spain). He is a full professor, researcher, and coordinator of the Multisensor Systems and Pattern Recognition Research (GISM) Group at Pamplona University. Has experience on industrial automation and has acquired a high knowledge in data acquisition techniques, embedded systems, pattern recognition methods, and artificial intelligence. Has worked in different projects related to multisensory perception actually, specifically about the development of electronic nose and electronic tongue systems applied in the industry and health sector. He has been the coordinator of two Colciencias projects (e.g.,Gastric Cancer detection of Colombian Patients) and currently has been involved in different european projects H2020 such as: Tropsense (2015) bTB-Test (2018) and Vogas (2019). The above has allowed publishing a huge number of papers in different applications. https://orcid.org/0000-0002-5241-2950

Ramón Ovidio García-Rico received his B.S. in Microbiology from the Pamplona University (Colombia), where he was a researcher and occasional teacher during the period 1998-2000. Through a grant from AECI (Spanish International Cooperation Agency) conducted his doctoral thesis. He received his Ph.D. in Molecular Biology and Biotechnology from the León University (Spain). He became a full-time lecturer of the Department of Microbiology, University of Pamplona (Colombia) and that same year he became Director of the Department. During the years 2007-2008, he was Dean of the Faculty of Basic Sciences. Subsequently, was representative of the professors to the Academic Council (2010-2011). Currently, he is the director of the Research Group in Microbiology and Biotechnology (GIMBIO). Professor Garcia-Rico has participated in the formulation and execution of interdisciplinary research projects at the national and international levels. https://orcid.org/0000-0002-2451-7958