

An Electronic Microtongue Based on a BDD Electrochemical Microcell for Qualitative Analysis of Domestic and Hospital Wastewaters

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Received: 31 December 2012 /Accepted: 10 August 2013 /Published: 26 May 2014

Abstract: An electronic microtongue using an electrochemical microcell was designed to monitor wastewater quality. The planar electrochemical microcells were micromachined in a microcrystalline Boron Doped Diamond (BDD) thin layer using a femtosecond laser. The electrochemical measurements with these BDD microcells were conducted using Differential Pulse Anodic Stripping Voltammetry (DPASV) for the major detection of heavy metal ions. Global signal obtained for raw wastewater (influent of Waste water Treatment Plant (WWTP)) and for water from Arve river were found much higher than those of treated wastewater (effluent of WWTP) and of treated wastewater from hospital. The DPASV signals have been processed by Principal Component Analysis (PCA) and K-Nearest Neighbours (K-NN). According to PCA results, waters were identified and discriminated with 99.75 % of the total variance of the dataset. Using K-NN with cross-validation approach, a good performance of the developed model was achieved for K=1 and 100 % of the samples were able to be classified in their original groups. Copyright © 2014 IFSA Publishing, S. L.

Keywords: Electronic microtongue, BDD electrode, DPASV, Pattern recognition methods, Wastewater analysis.

1. Introduction

Wastewater treatment is an important step in ensuring human and environmental health. It is regulated in different ways in various countries, the common goal being to minimize the pollution introduced into natural water bodies. However, the

analysis of wastewaters requires sophisticated techniques able to monitor every treatment stage. Amongst these techniques, electronic tongues take, in the last decade, an important place in water and wastewater quality monitoring. Commonly an electronic tongue (i.e., taste sensor) comprises a set of individual sensors with a broad and partially

overlapping selectivity towards compounds present in an aqueous sample [1]. The sensor array is coupled to an appropriate pattern recognition model capable of getting information from complex signals. Electronic tongues have exploited different measuring principles based on electrochemical techniques such as potentiometry [2, 3] or amperometry [4, 5]. Moreover, stripping voltammetry methods especially Differential Pulse Anodic Stripping Voltammetry (DPASV) has been proved feasible in wastewater trace metal measurements [6]. These techniques consist of two distinct steps. Firstly, in a preconcentration step, the analyte is accumulated from solution onto a suitable working electrode surface, which can be achieved either by electrochemical deposition or by physical adsorption depending on the nature of the analyte. In a second step, the potential of the working electrode is then adjusted in such a way that the analyte is liberated as ions from the electrode, with a resulting faradic current allowing a direct quantification of the amount of present metallic specie.

Recently, sophisticated sensors such as Boron-Doped Diamond (BDD) [7] and also Diamond-Like-Carbon (DLC) [8] have been elaborated in an integrated sensor array and can mimic the electronic tongue principle when their signals are subjected to suitable chemometrics. Electrodes made of BDD are extensively investigated for environmental and electroanalytical applications, because of their analytical properties, as low background current and a wide potential window, corrosion stability in aggressive media and resistance to biofouling. Moreover, BDD generally exhibits better figures of merit when compared to other carbon based electrodes [9, 10]. Furthermore, simultaneous detection of heavy metals is one of the most important potentialities of BDD electrodes. In fact, several heavy metals can be captured simultaneously without requiring a set of sensors. In this context, a new laser machined BDD planar microcells in conjunction with chemometric methods, such as Principal Component Analysis (PCA) and K-Nearest Neighbours (K-NN) were designed to build up an electronic microtongue aimed at monitoring various stages of domestic and hospital wastewater treatments, the electrochemical measurements being conducted using DPASV electrochemical technique.

2. Materials & Methods

2.1. Water Origins

Surface water was sampled in Arve River, downstream the Bellecombe Wastewater Treatment Plant (WWTP), domestic wastewater (influent & effluent) in WWTP of Belleville and the hospital wastewater in the hospital of Anamasse-Bonneville near Geneva (Fig. 1). The hospital is located near the

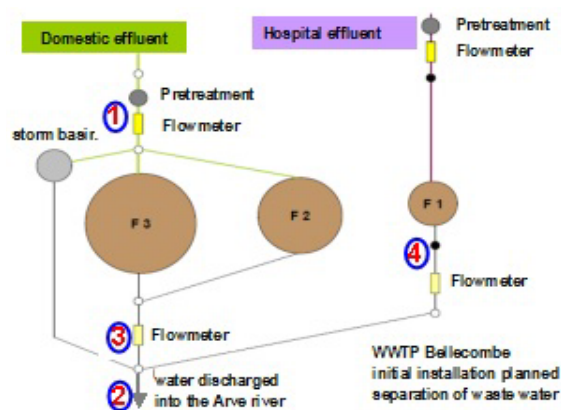


Fig. 1. Domestic and hospital wastewater origin: (1) Raw wastewater from Bellecombe WWTP, (2) Freshwater sample from Arve river, downstream the Bellecombe WWTP, (3) Treated wastewater from Bellecombe WWTP and (4) Treated wastewater from hospital. F1, F2 and F3 represent different treatment circuits with different throughputs in Population Equivalents.

Bellecombe WWTP. This is an experimental site of particular interest to achieve a specific research program (www.sipibel.org) to answer questions related to the characterization, treatability and impacts of hospital effluents. The site has a new hospital with a network of collection of individualized wastewater, where a processing line can be fully dedicated to the program of study over several years, a discharge in the Arve River which feeds a part of water resources for human consumption of Geneva. Therefore, a total of 20 samples of different wastewater treatment stages were analyzed. Samples were picked up from their origin localization in March 2012.

2.2. Reagents

Sulphuric acid (H_2SO_4) 95-97 % and hydrogen peroxide (H_2O_2) 30 % used for cleaning, and potassium citrate used in buffer were provided from Sigma-Aldrich (l'Isle d'Abeau Chesnes, France). Nitric acid (HNO_3) 68 % and hydrochloric acid (HCl) 37 % were provided from VWR international (Fontenay-sous-Bois, France).

2.3. BDD Electrode Fabrication

Electrochemical microcells were made of a film of 300 nm of Boron-Doped microcrystalline Diamond (BDD) deposited on an insulated silicon wafer of 4" in diameter provided from Neocoat company (La Chaux-de-Fonds, Switzerland). 300 nm thick polycrystalline Boron-Doped Diamond with boron concentration higher than 8000 ppm was grown by MPECVD on silicon coated with an insulating layer of silicon oxide and silicon nitride ($\text{Si}/\text{SiO}_2/\text{Si}_3\text{N}_4$) of 0.5 μm thickness, were purchased.

The 3 electrodes, working electrode (1 mm diameter), counter electrode and pseudo-reference electrode, were cut up from the BDD wafer by micromachining. This one was conducted by MANUTECH USD using a femtosecond laser (5 kHz, 2.5 W, 800 nm, 150 fs); a scanner head; a set of XYZ moving plates. The parameters used during processing were: power 150 mW; optic scanner 80 mm and speed 10–20 mm/s. The microcell resulting from laser machining is shown in Fig. 2. The working electrode is in the centre, around it is the “reference”, and the counter electrode is on the edge. Instead of the conventional saturated calomel electrode, the device used a pseudo-reference made of BDD [11].

2.4. Apparatus

A PalmSens sensor PC interface (Eindhoven, The Netherlands) was used to apply differential pulse voltammetry to the microcell. It was connected to a PC computer loaded with specific software. The flow cell was a 5 μ L cell made of PEEK, provided by BVT Technologies (Brno, Czech Republic). An O-ring seal defined the measuring volume, and the electrical contacts were obtained by pressure on the front side of the BDD electrodes.

2.5. Measuring Conditions

Prior to the experiments and after each calibration concentration, the BDD microcells were cleaned in a mixture of H_2SO_4 (95–97 %)/ HNO_3 (68 %) [V:V = 3:1] at 200–215 $^\circ\text{C}$ for 1.5 h, subsequently heated to 80 $^\circ\text{C}$ for 15 min in a mixture of H_2O_2 (30 %)/ ammonia (25 %) [V:V = 1:1] and finally ultrasound cleaned in distilled water, then in ethanol and finally dried with nitrogen.

Daily, BDD microcells had to be cleaned and activated in 10 mL of Piranha solution, a mix of H_2SO_4 (95–97 %)/ H_2O_2 (30 %) [V:V = 7:3] for 5 min. [Handle with care.] BDD microcells were then rinsed with distilled water, dried under nitrogen flow, and activated by cyclic voltammetry in 0.1 M HNO_3 . Finally, DPASV was used for all determinations. Instrumental parameters and standard measuring conditions were performed as follow: deposition potential and time were –2 V and 5 s respectively; start and end potentials were –2 and 0.5 V respectively, and scan rate 50 mV/s.

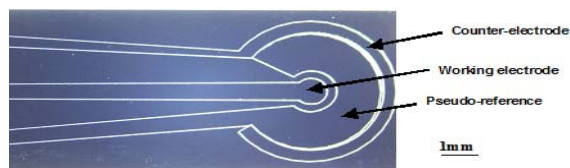


Fig. 2. The three electrodes cut up from the BDD wafer by laser-machining, the working electrode is in centre, then around it the "reference", and finally the counter electrode on the edge.

2.6. Feature Extraction

The features used for data analysis were extracted from the DPASV voltammograms of the BDD sensor. DPASV signals contain hundreds of measurements and usually overlapping regions with non-stationary characteristics. Thus, their high complexity should be reduced in order to avoid redundancies in the information and achieve proper mathematical models with correct generalization ability [12, 13]. In other words, by retrieving particular information from the original voltammogram, extracted features might be obtained that can confer more selectivity to BDD sensors. Several strategies have been previously reported for the feature extraction from the sensor response [14, 15]. Basically, they consist of either choosing directly amongst the voltammograms points [16, 17] or to compute new variables such as the Fast Fourier Transform (FFT) [18] or the Discrete Wavelet Transform (DWT) [19] to extract important features. Here, we opted for extracting specific features amongst the sensor response points. To achieve this aim, not only the traditional potentials and peak intensities but also additional features that can better characterize the sensor and analysis system were used. Hence, four representative features from the DPASV voltammograms were extracted, such as the potential of the major peaks, their maximum intensity, their half height width and finally their area.

Extracting the four aforementioned features from BDD voltammogram was an automated process via a written-in-house MATLAB 7.0.1 program.

2.7. Pattern Recognition Methods

The main objective of using pattern recognition methods in this particular application is to evaluate the performance of the electronic microtongue at identifying and discriminating domestic and hospital wastewaters. Performance is assessed by employing both unsupervised and supervised methods.

PCA pattern recognition technique is a powerful unsupervised method often employed with gas and liquid sensor arrays [20, 21]. The main objective of PCA consists of expressing information by a lower number of variables called principal components [22, 23]. These principal components are linear combinations of the original response vectors. The principal components are chosen to contain the maximum data variance and to be orthogonal. PCA allows reduction of multidimensional data to a lower dimensional approximation, while simplifying the interpretation of the data by the first two or three principal components (PC1, PC2 and PC3) in two or three dimensions and preserving most of the variance in the data.

K-NN is a non parametric supervised learning algorithm that has found wide usage in pattern recognition in gas and liquid application

fields [24, 25]. Basically, it assumes that similar patterns are assigned as belonging to one class based on the notion of distance. Although the Euclidean metric distance is the one commonly used, other metric distance can also be used (i.e., Chebyshev, Mahalanobis, Minkowski and Hamming). K -NN classifies an unknown test sample by finding the K nearest neighbours in the training set using a metric distance and assigning the label of that class represented by a majority among the K neighbours [26, 27]. The choice of K is optimized by calculating the prediction ability with different K values. Small K values are frequently preferred. If $K=1$, so the algorithm is simply called the nearest neighbour algorithm.

PCA and K -NN were performed using the MATLAB 7.0.1 software (MathWorks Inc., Natick, Massachusetts, USA).

3. Results and Discussion

3.1. DPASV Voltammograms

The first set of DPASV voltammograms, clearly shows the effect of wastewater treatment on the signal from the electronic microtongue (before and after wastewater treatment, Fig. 3). Indeed, in the treated wastewater, we have observed a significant decrease in the major peaks present in the untreated water.

However, the voltammograms of the treated domestic and hospital wastewater are apparently very similar. Finally, the voltammogram of the waters of the Arve river seems closer to untreated wastewater than treated ones (Fig. 3 (n 1) and Fig. 4 (n 2)).

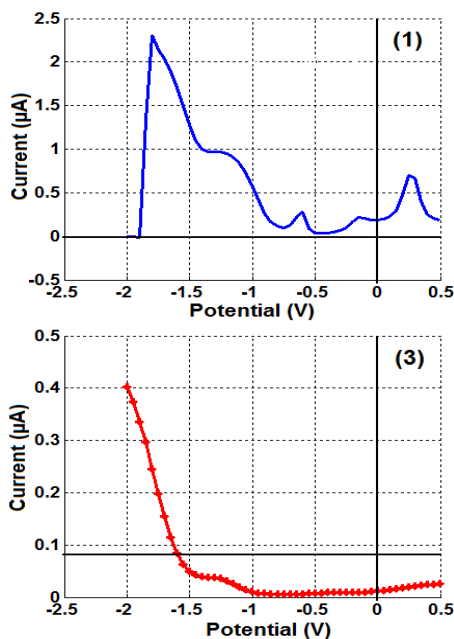


Fig. 3. DPASV obtained with the BDD micromachined microcell on (1) Raw wastewater from Bellecombe WWTP and (3) Treated wastewater from Bellecombe WWTP.

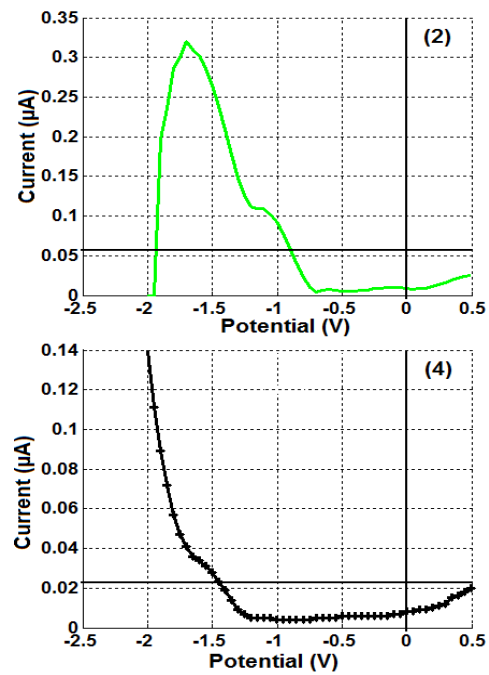


Fig. 4. DPASV obtained with the BDD micromachined microcell on (2) Freshwater sample from Arve river, downstream the Bellecombe WWTP and (4) Treated wastewater from hospital.

3.2. PCA Results

The outcomes of the set of wastewater experiments were evaluated using PCA and K -NN, as introduced in Section 2.6.

The PCA score plot was used as an exploratory technique to investigate clustering of data points within the multi-dimensional space of water features. To achieve this aim, four characteristic features were obtained from the DPASV voltammograms for each wastewater. These variables were organized in a rectangular matrix as a database. A mean-centering pre-processing technique was applied to the dataset. Fig. 5 shows the projections of the experimental results on a three-dimensional (3D) space formed by the first three principal components (PCs). A value of 94.92 % data variance explained by the first two PCs and of 4.83 % of data variance explained by the third PC indicates their importance for pattern separation. This means that the differences existing among samples along the first two axes are more significant than those existing along the third axis.

However, the third PC contributes to the discrimination between freshwater from Arve river and treated wastewater from hospital. Raw wastewater from Bellecombe and treated wastewater from Bellecombe WWTP can be easily discriminated in the PCA plot. In summary, the first three PCs explained 99.75 % of the total variance in the wastewaters data set. The PCA score plot showed that a clear discrimination was possible between all the wastewaters under study.

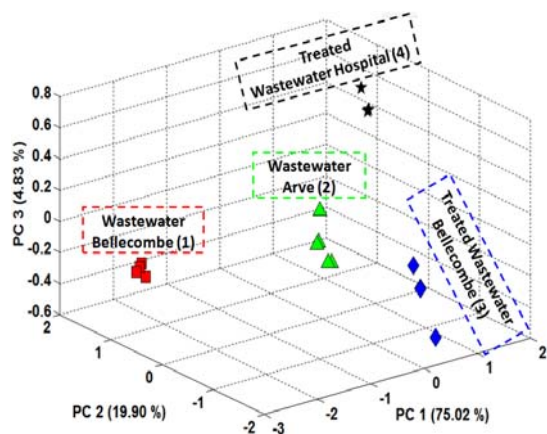


Fig. 5. 3D-score plot of PCA performed from BDD extracted features. A total of 20 samples were analyzed. As can be observed, a correct discrimination is obtained for the different wastewaters: (1) Raw wastewater from Bellecombe WWTP, (2) Freshwater sample from Arve river, downstream the Bellecombe WWTP, (3) Treated wastewater from Bellecombe WWTP and (4) Treated wastewater from hospital.

3.3. K-NN Results

Supervised classification was performed in order to test the electronic microtongue capability to identify domestic and hospital wastewater samples. The choice of K is optimized by calculating the prediction ability with different K values and we have found out that K -NN with $K=1$ is the best choice for our application since a superior classification rate was reached. The distance criterion used in the present study was the Euclidean distance. Initially, the classification model was constructed in which all the wastewater samples were used as the training set. In a second stage, to validate the classification model thus obtained and its stability in predicting, a 4-fold cross-validation step was performed (the samples were split into 4 groups, each of them containing 25 % of the total). In order to perform this cross-validation procedure, 3-folds were used for training and the last fold was used for test. The same process was performed four times with four different training and prediction sets, ensuring that all the samples were included in the prediction set. The classification rate is given by the average rate over the 4-folds.

Table 1 reports the confusion matrix, which means the true class value against the class value predicted by the electronic microtongue; the values are given in percentage and are obtained by averaging over the four test sets. It may be seen that for all the wastewater clusters the percentage of correctly predicted samples of the test set was 100 %.

Conclusions

This work focused on the challenging task to develop an electronic microtongue based on BDD

sensors coupled with a DPASV electrochemical method in order to create a tool capable of discriminating domestic wastewaters from hospital ones. The planar electrochemical microcells were micromachined in a BDD microcrystalline thin layer using a femtosecond laser. The data collected from DPASV voltammograms were pre-processed by extracting relevant information. A clear difference was found between hospital and domestic wastewaters by DPASV voltammograms and data analysis. PCA and K -NN were used for the qualitative classification. PCA results showed satisfactory discrimination amongst the tested wastewaters and K -NN with cross-validation approach reached 100 % success rate in the recognition of the analyzed wastewaters.

The electronic microtongue developed can constitute the base of new field monitoring devices paving the way to qualitative in situ measurements. Work is underway to transform this electronic tongue in an in situ quantification tool of metals.

Table 1. K -NN Classification results of wastewaters: (1) Raw wastewater from Bellecombe WWTP, (2) Freshwater sample from Arve river, downstream the Bellecombe WWTP, (3) Treated wastewater from Bellecombe WWTP and (4) Treated wastewater from hospital.

Actual	Predicted			
	(1)	(2)	(3)	(4)
(1)	100 %			
(2)		100 %		
(3)			100 %	
(4)				100 %

Acknowledgements

This work has been funded in part by AUF PCSI under project N°: 6313PS025 and PHC Maghreb N° 14 (TASSILI Project N° 12 MDU881 M; VOLUBILIS Project N° 27960 UG; UTIQUE Project). Authors thank SIPIBEL program for providing water samples.

References

- [1]. K. Toko, Taste sensor, *Sensors and Actuators B*, Vol. 64, 2000, pp. 205–215.
- [2]. L. Lvova, E. Martinelli, E. Mazzone, A. Pedè, R. Paolesse, C. Di Natale, A. D'Amico, Electronic tongue based on an array of metallic potentiometric sensors, *Talanta*, Vol. 70, 2006, pp. 833–839.
- [3]. P. Ciosek, W. Wroblewski, Miniaturized electronic tongue with an integrated reference microelectrode for the recognition of milk samples, *Talanta*, Vol. 76, 2008, pp. 548–556.
- [4]. M. Scampicchio, D. Ballabio, A. Arecchi, S. M. Cosio, S. Mannino, Amperometric electronic tongue for food analysis, *Microchimica Acta*, Vol. 163, 2008, pp. 11–21.

- [5]. L. Pigani, G. Foca, K. Ionescu, V. Martina, A. Ulrici, F. Terzi, M. Vignali, C. Zanardi, R. Seeber, Amperometric sensors based on poly (3,4-ethylenedioxythiophene)-modified electrodes: Discrimination of white wines, *Analytica Chimica Acta*, Vol. 614, 2008, pp. 213–222.
- [6]. A. Sbartai, P. Namour, A. Errachid, J. Krejci, R. Šejnohová, L. Renaud, M. Larbi Hamlaoui, A.-S. Loir, F. Garrelie, C. Donnet, H. Soder, E. Audouard, J. Granier, N. Jaffrezic-Renault, Electrochemical Boron-Doped Diamond Film Microcells Micromachined with Femtosecond Laser: Application to the Determination of Water Framework Directive Metals, *Analytical Chemistry*, Vol. 84, 2012, pp. 4805–4811.
- [7]. N. Jaffrezic-Renault, A. Sbartai, A. Errachid, L. Renaud, P. Namour, A.-S. Loir, F. Garrelie, C. Donnet, H. Soder, E. Audouard, J. Granier, Procédé de fabrication de microcellules électrochimiques planaires, *French Patent*, No 1250807, 2012.
- [8]. B. Khadro, A. Sikora, A.-S. Loir, A. Errachid, F. Garrelie, C. Donnet, N. Jaffrezic-Renault, Electrochemical performances of B doped and undoped diamond-like carbon (DLC) films deposited by femtosecond pulsed laser ablation for heavy metal detection using square wave anodic stripping voltammetric (SWASV) technique, *Sensors and Actuators B*, Vol. 155, 2011, pp. 120–125.
- [9]. R. Trouillon, D. O'Hare, Comparison of glassy carbon and boron doped diamond electrodes: Resistance to biofouling, *Electrochimica Acta*, Vol. 55, 2010, pp. 6586–6595.
- [10]. E. A. McGaw, G. M. Swain, A comparison of boron-doped diamond thin-film and Hg-coated glassy carbon electrodes for anodic stripping voltammetric determination of heavy metal ions in aqueous media, *Analytica Chimica Acta*, Vol. 575, 2006, pp. 180–189.
- [11]. A. Sbartai, Ph. Namour, N. Sarrut, J. Krejci, R. Kucerova, M. L. Hamlaoui, N. Jaffrezic-Renault, Direct detection of lead in RTIL using DPASV on BDD film microcells and determination of concentration factor after extraction from aqueous samples, *Journal of Electroanalytical Chemistry*, Vol. 686, 2012, pp. 58–62.
- [12]. F. Winquist, Voltammetric electronic tongues – basic principles and applications, *Microchimica Acta*, Vol. 163, 2008, pp. 3–10.
- [13]. E. Richards, C. Bessant, S. Saini, Multivariate data analysis in electroanalytical chemistry, *Electroanalysis*, Vol. 14, 2002, pp. 1533–1542.
- [14]. P. Ciosek, Z. Brzózka, W. Wróblewski, E. Martinelli, C. Di Natale, A. D'Amico, Direct and two-stage data analysis procedures based on PCA, PLS-DA and ANN for ISE-based electronic tongue—effect of supervised feature extraction, *Talanta*, Vol. 67, 2005, pp. 590–596.
- [15]. L. A. Dias, A. M. Peres, A. C. A. Veloso, F. S. Reis, M. Vilas-Boas, A. A. S. C. Machado, An electronic tongue taste evaluation: Identification of goat milk adulteration with bovine milk, *Sensors and Actuators B*, Vol. 136, 2009, pp. 209–217.
- [16]. Z. Haddi, A. Amari, B. Bouchikhi, J. M. Gutiérrez, X. Cetó, A. Mimendia, M. del Valle, Data Fusion from Voltammetric and Potentiometric Sensors to build a Hybrid Electronic Tongue applied in Classification of Beers, *American Institute of Physics (AIP) Conf. Proc.*, 1362, 2011, pp. 189–190.
- [17]. K. Sghaier, H. Barhoumi, A. Maaref, M. Siadat, N. Jaffrezic-Renault, Characterization and Classification of Groundwater from Wells Using an Electronic Tongue (Kairouan, Tunisia), *Journal of Water Resource and Protection*, Vol. 3, 2011, pp. 531–539.
- [18]. D. Calvo, A. Duran, M. del Valle, Use of sequential injection analysis to construct an electronic-tongue: Application to multidetermination employing the transient response of a potentiometric sensor array, *Analytica Chimica Acta*, Vol. 600, 2007, pp. 97–104.
- [19]. X. Cetó, J. M. Gutiérrez, M. Gutiérrez, F. Céspedes, J. Capdevila, S. Mínguez, C. Jiménez-Jorquera, M. del Valle, Determination of total polyphenol index in wines employing a voltammetric electronic tongue, *Analytica Chimica Acta*, Vol. 732, 2012, pp. 172–179.
- [20]. Z. Haddi, A. Amari, H. Alami, N. El Bari, E. Llobet, B. Bouchikhi. A portable electronic nose system for the identification of cannabis-based drugs, *Sensors and Actuators B*, 155, 2011, pp. 456–463.
- [21]. J. M. Gutiérrez, Z. Haddi, A. Amari, B. Bouchikhi, A. Mimendia, X. Cetó, M. del Valle, Hybrid electronic tongue based on multisensor data fusion for discrimination of beers, *Sensors and Actuators B*, Vol. 177, 2013, pp. 989–996.
- [22]. L. Gil, J. M. Barat, D. Baigts, R. Martínez-Mañez, J. Soto, E. Garcia-Breijo, M.-C. Aristoy, F. Toldrá, E. Llobet, Monitoring of physical–chemical and microbiological changes in fresh pork meat under cold storage by means of a potentiometric electronic tongue, *Food Chemistry*, Vol. 126, 2011, pp. 1261–1268.
- [23]. A. Amari, N. El Bari, B. Bouchikhi, Conception and development of a portable electronic nose system for classification of raw milk using principal component analysis approach, *Journal Sensors & Transducers*, Vol. 102, 2009, pp. 33–44.
- [24]. M. Falasconi, E. Gobbi, M. Pardo, M. D. Torre, A. Bresciani, G. Sberveglieri, Detection of toxigenic strains of *Fusarium verticillioides* in corn by electronic olfactory system, *Sensors and Actuators B*, Vol. 108, 2005, pp. 250–257.
- [25]. Q. Chen, J. Zhao, S. Vittayapadung, Identification of the green tea grade level using electronic tongue and pattern recognition, *Food Research International*, Vol. 41, 2008, pp. 500–504.
- [26]. D. S. Dhaliwal, P. S. Sandhu, S. N. Panda, Enhanced K-Nearest Neighbor Algorithm, *World Academy of Science, Engineering and Technology*, Vol. 49, 211, pp. 681–685.
- [27]. P. Ciosek, W. Wroblewski, The analysis of sensor array data with various pattern recognition techniques, *Sensors and Actuators B*, Vol. 114, 2006, pp. 85–93.