Detection of microbial contamination in potable water by Nanowire technology

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Abstract— ***It is well known that the lack of control and sanitation of water in developing countries has cause very significant epidemiological events. In the last decades the situation of water supplies and sanitation has improve all over the world. Despite of it, in the European Union there are a considerable number of confirmed cases of water-borne infections even though the restrictive law. Electronic Noses (ENs) has shown to be a very effective and fast tool for monitoring microbiological spoilage and quality control. The aim of this study was test the ability of a novel EN for the detection of bacterial presence in potable water in cooperation with analytical (pH) and optical (photometer) techniques. The achieved results notably advocate the use of EN in industry laboratories as a very important tool in water quality control.***

Keywords-component; Nanowire sensors; water; microbial contamination; Electronic Nose.

I. INTRODUCTION

Water is one of the simplest chemical compounds, normally is a liquid at standard pressure and temperature, although it can co-exist with solid and gaseous state.

On the earth's surface water covers 71 %, and it's vital for all known forms of organisms. From that percentage just the 2.5% is freshwater, generally characterized by having low concentrations of total dissolved solids and dissolved salts, and the 98.8% is in ice and groundwater. Less than 0.3% of all freshwater is in rivers, lakes, and the atmosphere. Surprisingly just the 0.003% is contained in biological structures and manufactured products. Around the 70% of the fresh water used by humans goes to agriculture.

Even though it provides no calories or organic nutrients is essential to humans and any kind of life. Access to safe drinking water has been improved over the last decades in almost every part of the world, but approximately one billion people still lack access to safe water and over 2.5 billion suffer the absence to adequate sanitation. There is a clear correlation between the availability to safe water and gross domestic product per capita. The data published by UNIDO affirm that: 1.4 million children die from water borne disease caused by lack of adequate sanitation every year, in average one child each 20 seconds in developing countries.

Despite of the restrictive and accurate controls established by law in the European Community every year are reported events of water-borne diseases. Some of the most common microbiological contaminants are *Escherichia coli, Listeria monocytogenes, Salmonella typhimurium and Pseudomonas fluorescens*. The ECDC (European Center of Control Disease) recalls that around 9500 cases of *E. coli* toxins producing strains were confirmed in 2013. This microorganism belongs to the group of Coliforms. This group is used as the most common microbial contamination indicator in water but in particular *E. coli* is used also as indicator of fecal contamination. The infection may lead in the worst cases to fatal hemolytic uremic syndrome (HUS) affecting the renal system and requiring hospital care.

Even if pure H2O is tasteless and odorless, can dissolve many different substances, acquiring a wide range of tastes and odors. Mammals, and other animals, have developed senses that enable them to evaluate the portability of water by avoiding water that is too salty or putrid [1].

Current techniques used in the quality control of water requires time, a substantial amount of money and in most of cases requires specific skills [2].

The assay performed by Electronic Nose (EN) equipped with nanowire tech is based in the analysis of the head space. The Nanowires, Fig. 1, has an extraordinary length-to-width ratio and a singular crystalline quality, resulting in enhanced sensing performances as well as long-term stability for sustained operation. In addition the 3 dimensional network formed by the nanowires increment in a big range the adsorption surface, enhancing the response of the instrument decreasing the threshold [3].

The EN equipped with nanowire technology is able to reveal the present of the microorganisms trough the detection of the organic volatile compounds produced during their metabolic activities. In some cases, individualizing species of the same group of microorganisms [4][5].

The aim of this work was the establish a new, rapid and economic fouling, based on the cooperation between the use of a novel EN equipped with nanowire tech, analytical (pH) and

optical (photometer) techniques, for the detection of bacterial presence in water [6].

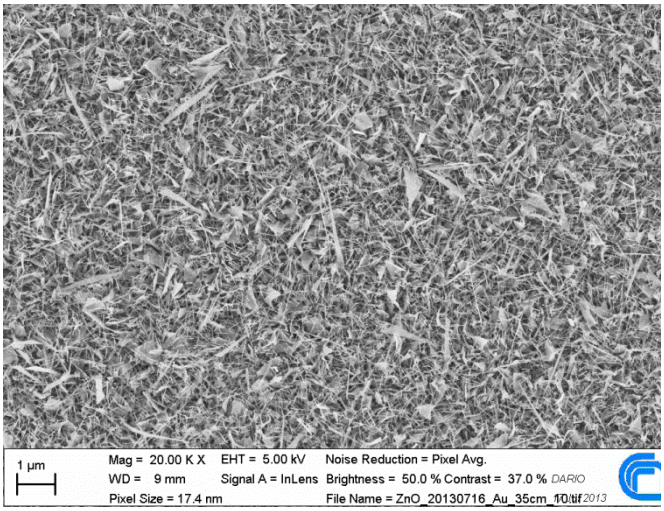

Figure 1. SEM image of ZnO nanowires synthetized by PVD.

II. MATERIALS & METHODS

A. Sample preparation

E. coli cultures were developed in 7 ml of Brain Heart Infusion (BHI) (OXOID) liquid media [7]. This strain was provided by the UMCC (Unimore Microbial Culture Collection) . Once inoculated the tubes were incubated at 30º C during 48 h. Subsequently the cultures were centrifuged at 3000 rpm during 10 min. The supernatant was discarded and the cells were re-suspended in 1.5 ml of sterilized tap water. A total volume of 400 ml of sterilized tap water was inoculated with the suspension of cells prepared before until it reaches the same turbidity as the third standard of the McFarland series. These standards are used as a reference to adjust the turbidity of bacterial suspensions in order to have a number of bacteria within a given range. Number 3 of McFarland standard corresponds with a bacterial concentration of 9 x 10^8 CFU/ml. Other 2 dilutions were prepared as well, 400 ml of sterilized tap water inoculated with 9 x 10^5 CFU/ml and 400 ml of sterilized tap water inoculated with 9 x 10^2 CFU/ml. Once prepared all three dilutions were incubated at 30 ºC during the 5 days of analysis (T0, T1, T2, T3 and T4).

B. pH

A control of the pH was done for all the three dilutions. The pH was measured each hour during the first 24 h of analysis and then every 12 h. During the grow of *E. coli* organic acids are released to the environment where it grows. For that reason the control of pH is an optimal indicator of the microbial presence in water.

C. Photometer

To survey the development of the microorganisms in water it was measured the Optical Density (OD) at 600 nm for all three dilutions. The OD data were taken each hour during the first 24 h of analysis and then every 12 h to follow the same procedure as in the case of the pH.

D. Electronic Nose

The used instrument to perform the analysis was the EN EOS835 (SACMI IMOLA scarl, Imola, Italy). It's furnish with a 20 ml internal volume thermally controlled sensor chamber were are placed 6 MOX gas sensors. Four of these sensors were prepared with the RGTO thin film technology [8], and the other two were constructed with nanowire technology [9]. Even though the EN EOS835 is on the market the set of sensors has been modified in cooperation with Sensor Lab CNR INO Brescia Italy. It was also equipped with an auto-sampler headspace system HT200 (HTA srl, Brescia, Italy), supporting a 40 loading sites carousel and a shaking oven to equilibrate the sample headspace. A volume of 2 ml was placed separately for every dilution in a sterilized chromatographic vial of 20 ml. Once inoculated all the vials were cover with an aluminum crimp, a coated PTFE/silicon septum and crimped. In every carousel 4 of the 40 loading positions were dedicated to vials containing 500 µl of butanol. This compound act as the internal standard of the instrument. This operation was repeated during the 5 days of analysis (from T0 to T4).

The vials for every dilution were placed in a randomized mode into the HT200 carousel. Each vial was incubated at 40°C for 10 min into the HT200 oven, by shaking it during all the incubation in order to reach the equilibrium of the head space. The sample headspace (4 ml) was then extracted from the vial in static headspace path and injected into the carrier flow (speed 4 ml min) through a properly modified gas chromatography injector (kept at 40°C to prevent any condensation). Using synthetic chromatographic air with a continuous flow rate of 10 ml/min performed the sensor baseline and the recovery time was 28 min [10].

Principal Component Analysis (PCA) performed explorative data analysis. Data were processed by EDA software, a home written software developed in MATLAB® at Sensor Laboratory [11]. Feature extraction is usually unsupported, the most common examples being Principal Component Analysis. PCA can, by itself, transfer a good visual idea of the interclass split-up; in fact, this is how it is usually applied. The goal was to obtain a good visual separation of the clusters, not just to have high estimate accuracy. To this end, PCA was systematically used to judge the clusters separation.

III. RESULTS & DISCUSSION

Regarding to the OD obtained results (Fig. 2) it is possible to observe a typical microbial curve of growth in all three dilutions. The Lag phase goes from 0 to 8 h, then the inoculated cells start to divide and the OD increase until it reach the stationary phase at 20 h. A proportional but inverse response came out observing the pH data (Fig. 2).

As the microorganisms develop inside the water, the OD increases because the turbidity increases as well. The metabolic activities carried out by the microorganisms during their grow causes the production of a big amount of organic acids that subsequently decrease the pH values. Observing Fig. 2 it is possible to see that these two variables are inversely related so a test of correlation between pH and OD was performed for the 3 dilutions. In all three cases the negative correlation between the 2 variable was confirmed obtaining a $R^2 > 0{,}92$ (Fig 3.)

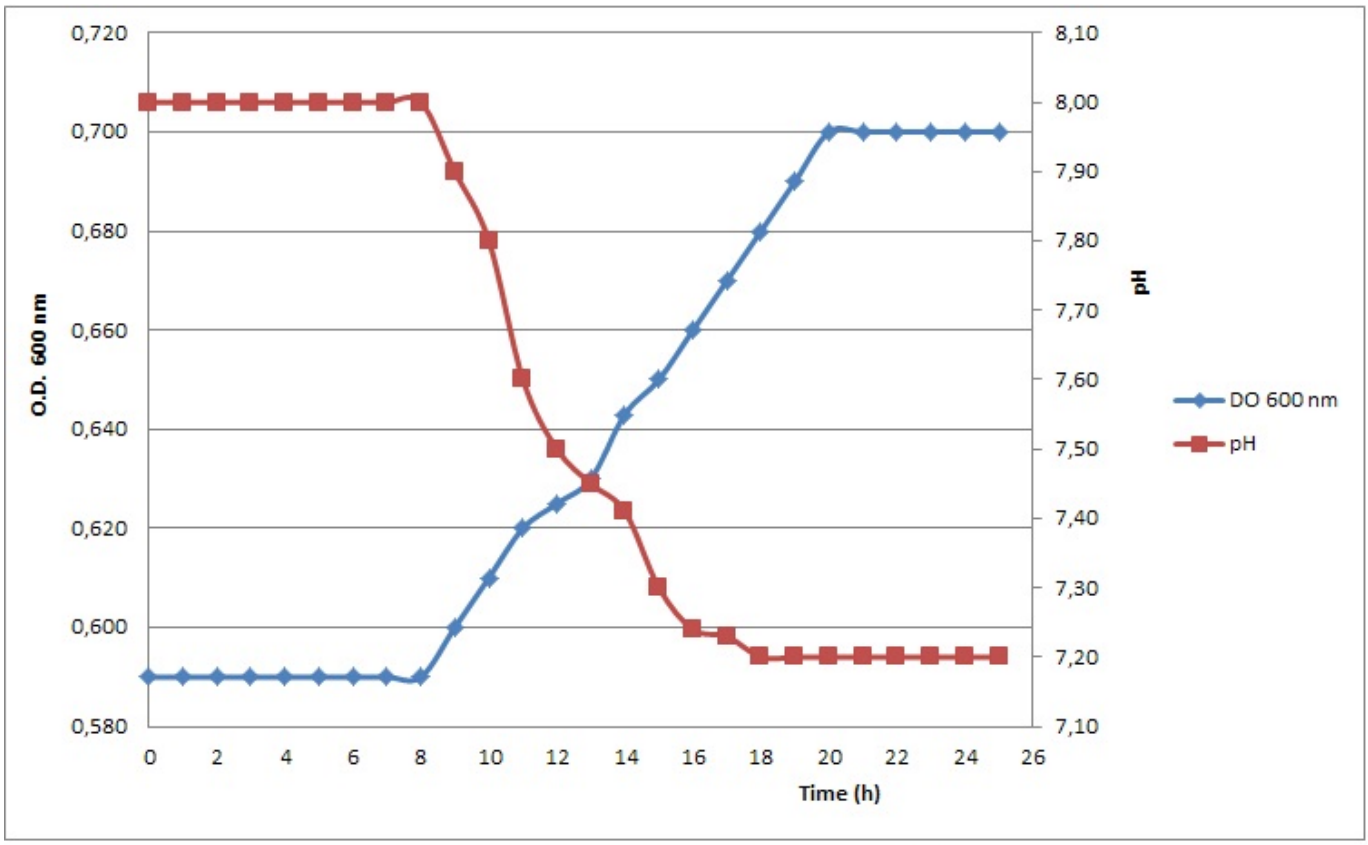

Figure 2. Obtained result for pH and OD for the dilution 9 x 10^2 E. coli

Concerning to the EN result the Fig. 4 is the PCA score plot formed by the samples that belongs to the first day of analysis and those analyzed in the last 48 h (T3 and T4) for all the three microbial concentrations. The continuous change in the head space of the samples is perfectly represented. The samples that belong to the first day of analysis (black stars) remains in the left part of the graphic while those that belongs to T3 (blue circles) and T4 (green triangles)are placed in the right side of the figure. Samples of T3 and T4 occupy the same space in the right part inside the PCA score plot. In this case, and then 96 h of analysis, the metabolic activities carried out by the microorganism are not very active due to the scarce concentration of metabolic resources that were present in the water. At this point the head space remains table and does not exhibit any changes also because the microorganisms does not divide anymore and start to dye.

On the other hand in Fig. 5 are represented the evolution of the samples with a microbial load of $9x10^2$ CFU/ml. The drift is the same as in the previous graph but in particular in this case the samples of the T3 (blue circles) forms a more compact cluster while those that belongs to the T4 are more dispersed. The concentration of resources was the same for all the three microbial loads. But in particular in this case the rate between microbial concentration and resources was enhanced so in average the concentration of available resources was increased respect the other two microbial loads. Because of this reason it is possible to observe a compact cluster of T3 samples. In this case the microorganisms inside the water culture are still able to divide and carry out some simple metabolic process. However samples of the T4 are distributed on the right part of the PCA, it can be due that at this time of analysis the microorganisms present in the samples are in the logarithmic decline phase of the microbial curve of grow.

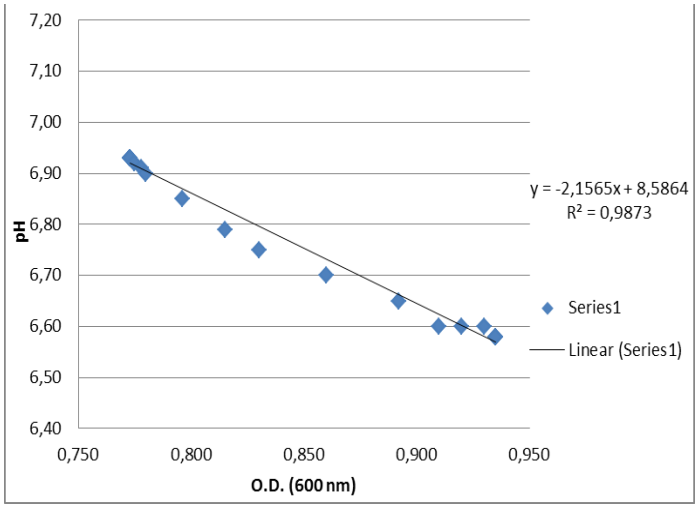

Figure 3. Linear correlation and R^2 value for the pH and OD data of the $9x10^8$ CFU/ml of *E. coli*.

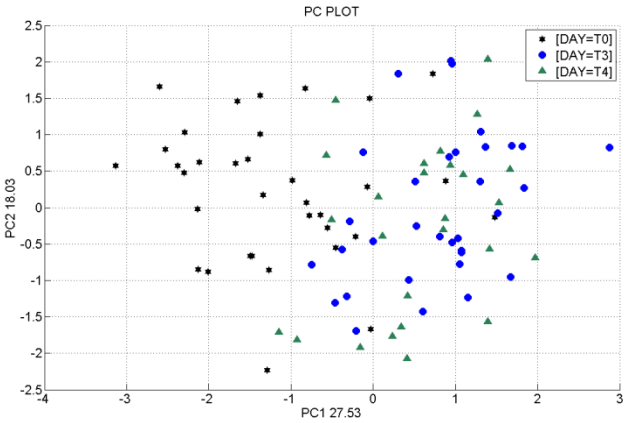

Figure 4. PCA score plot showing the T0, T3 and T4 of analysis for all three microbial loads.

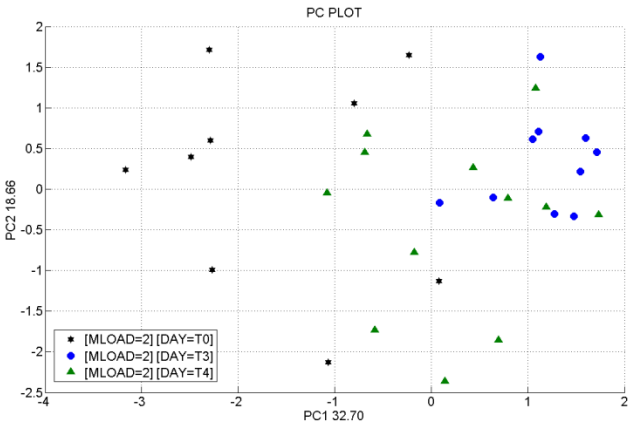

Figure 5. PCA score plot showing the T0, T3 and T4 of analysis for of the samples with a microbial load of 9x102 CFU/ml.

IV. CONCLUSIONS

This work attests that the EN, once trained, is a potential, economic and useful tool to be applied in the monitoring quality control of potable water. It provides a faster response than the conventional techniques. EN equipped with nanowire technology that enhance the threshold of the instrument, is able to detect the development of the microorganisms directly in water. As future work will be interesting correlate the CFU with the answer produce by the EN, decreasing the concentrations already used, to establish a threshold of the instrument an to test the response using different kinds of water and microorganisms [10][12].

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