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Microbial Fuel Cells as Effective Tools for Energy Recovery and Antibiotics Detection in Water and Food

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Abstract: This work demonstrates that microbial fuel cells (MFCs), optimized for energy recovery, can be used as an effective tool to detect antibiotics in water-based environments. In MFCs electroactive biofilms works as biocatalysts converting the chemical energy of organic matter, working as the fuel, in electrical energy. The efficiency of the conversion process can be significantly affected by the presence of contaminants acting as toxicants for the biofilm. In the present work, we demonstrate that MFCs can successfully detect residues of antibiotics in water and water-based electrolytes associated to the food industry, especially testing contamination in honey that can be used as a fuel. The effectiveness of MFCs to sense antibiotics is here demonstrated for tetracycline that was added to both water and water/honey electrolytes with a minimum concentration close to 3.5 μg/kg. MFCs not only efficiently detect the presence of tetracycline in both the electrolytes, but also recover the same performance after each cycle of exposure, showing to a be very robust and reliable technology for both biosensing and energy recovery.

Keywords: microbial fuel cells; energy recovery; biosensors; bio-electrochemical sensors; Antibiotic contamination

1. Introduction

Foods of animal source, as eggs, milk, honey and meat plays an important role in our ordinary diets and during the last years a rapid increase of their consumption was registered, having been demonstrated to be so useful for human health and well-being [1-3]. In this scenario an ever-increasing importance must be paid to the presence of veterinary medicines in foods, as denounced by the European Food Safety Authority (EFSA) [3]. Substances as hormones, antibiotics and growth promoters are extensively employed to prevent and treat diseases in animals, to boost their growth and improve feeding [1-3]. Unfortunately, several of them can be transferred as contaminants in food products [3]. One of the greatest concerns is regulating and controlling food safety, especially monitoring the presence of veterinary medicines in animal-source foods [1-3]. In this scenario, the World Health Organization (WHO) has identified antibiotic resistance as one of the three biggest threats to human health. Clear evidence has been provided of the adverse consequences for human health of the resistance to antimicrobials developed by the organisms because of the continuous exposure to antibiotics for non-human use [4-6]. Antibiotic resistance is a global sanitary emergence: the largest the amount of antibiotics used, the highest the probability for bacteria to develop resistance mechanisms to them. The result of this mechanism is that antibiotics become ineffective, causing substantial failure of antimicrobial treatments. For these reasons, awareness is increasing on the need and urgency to actively protect antibiotics against over-use [7,8]. To account all these issues, the development and employment of methods able to detect animal-drugs residues in animal-sources foods, helping to determine if their quantity is lower to the maximum residues limits (MRL), represent the great challenges in many countries to ensure a certain level of food safety [9,10].

Moreover, methods are needed that are capable to accurately reveal the presence of antibiotics continuously and on-site even in complex matrices, such as food and biological samples [11-16]. In this regard, in the literature several and different analytical methods are proposed, such as High-Performance liquid chromatography (HPLC) coupled with Mass Spectrometry (MS) and Liquid Chromatography combined with tandem mass spectrometry (LC-MS/MS), to detect antibiotics accurately and simultaneously in different environmental media [12,13]. Other determination techniques such as capillary electrophoresis (CE) [14], Raman Spectroscopy [15], and enzyme linked immunosorbent assays [16] were exploited to monitor drugs residues in food products. Even very innovative, many of the bioassay and conventional methods cannot be implemented for on-site routine use, since they need pretreatments, are expensive and not enough sensitive. With the main target to overcome all these limits, biosensors received great interest as effective methods for screening antibiotics residues. Biosensors not only offer high sensitivity and selectivity, but also ensure high degree of automation, combined to cost-effectiveness, real-time measurements and high throughput [17-19]. Basically, in the general scheme of biosensors, a biological recognition element interacts with the target compound inducing a biological response that the physical transducer transforms in a detectable signal proportional to the content of analytes [20-23]. Several biological recognition elements, including cofactors, enzymes, antibodies, organelles, tissues, cells, and whole microorganisms have been used in the design of biosensors [24]. Among these biological elements, microorganisms (e.g. algae, bacteria and yeast) offer a valid alternative to fabricate biosensors thanks to their easiness of manipulation, better viability and stability in vitro and ability to enhance performance of biosensors [25]. Nonetheless, further improvements are necessary to develop more effective biosensors, which can be promising tools for the detection and quantification of antibiotics in food products [11]. One of the most promising type of bio-electrochemical sensors, based on whole microorganisms as recognition elements, is represented by Microbial Fuel Cells (MFCs), which converts the chemical energy, contained in organic compounds, known as fuels, into electrical energy thanks to the metabolic activity of so called exo-electrogenic bacteria [26,27]. Since the power output is strictly correlated with the metabolic activity of these microorganisms, the presence of a toxicant can directly affect the overall performance of the device in terms of the output electrical parameters [28-32]. Therefore, in MFC-based biosensors, microbial metabolism enables the conversion of a chemical signal, associated to the energy trapped in the fuel, into an electrical signal, i.e. the output electrical energy of MFCs. Thus, the energy conversion and the signal transduction steps are intimately coupled, avoiding the need of any external transducer and additional power unit. MFC-based biosensors can be successfully used ad fast response early low maintenance detectors, being also cost effective, as they can be built on low-cost carbon-based materials [33,34].

In this work, air-cathode Single Chamber Microbial Fuel Cells (a-SCMFCs) are proposed as effective bio-electrochemical devices for the detection of a particular kind of antibiotic, tetracycline, added to a water-based electrolyte and to the honey, previously investigated as effective more complex electron donor [35]. Based on the results of our past article [35], optimal proliferation of microorganisms at the anode has been ensured using nanostructured anodes, based of carbon paper decorated by nanofibers made of polyethylene oxide (CP/PEO-NFs). This approach allowed to enhance not only the overall a-SCMFCs performance but also to improve their capability to monitor the presence of antibiotic into the electrolytes. We investigated a-SCMFCs as bio-electrochemical sensors for antibiotic detection by employing and comparing two different electrolytes. The first is a water-based electrolyte used as SCMFCs' behaviors benchmark when antibiotics are added in traces to water. We selected a very low concentration value of tetracycline, which is close to (3.53 ±0.13) μg⁄kg and refereed to the amount of honey. This value was selected since it results to be one order of magnitude lower than the MRL defined for honey as food matrix, which is close to 10 μg⁄kg. Proven/Demonstrated/Confirmed thus the effectiveness of SCMFCs as bio-electrochemical sensor for tetracycline detection when water-based electrolyte was employed, we investigated, in the present work, the possibility to add the antibiotic traces directly into honey-based electrolyte. Indeed, to this purpose, the second electrolyte was prepared dissolving honey to deionized water in quantities equal to those used for sodium acetate-based electrolyte. Finally, we demonstrated the SCMFCs' capability

to detect the presence of drugs directly into the food-matrix without the necessity to extract the antibiotics before their detection. It was possible to achieve a decreasing of current density values, close to 50%, when tetracycline was added to the water-based electrolyte. Moreover, 96% of decreasing of current density reached when (3.53 ±0.13) µg/kg of tetracycline was added to the electrolytes based on honey, demonstrating thus the possibility to apply SCMFCs as sensor for the tetracycline detection, directly using honey as carbon sources for microorganisms. Finally, we evaluated and confirmed the capability of SCMFCs to recover the same current density value when fresh electrolyte, based on only sodium acetate and honey, is employed. Finally, the behavior of all devices is also analyzed evaluating the amount of energy recovered (E_{rec}) by a unit volume of the electrolyte [36-38]. E_{rec} obtained for both electrolytes, based onto honey and sodium acetate with and without the presence of antibiotics, was compared, demonstrating the microorganisms' capability to accurately detect the presence of antibiotics, guaranteeing also their recovery when the antibiotic is not present into electrolytes.

2. Materials and Methods

2.1. Materials and Nanofibers Synthesis

With the aim to improve biofilm growth, we fabricated composite nanostructured anodes following our previous work [35]. Carbon Paper (CP, from Fuel Cell Earth, Woburn, Massachusetts, USA) was decorated with nanofibers (NFs) made of polyethylene oxide (PEO, Sigma Aldrich) with a molecular weight of 600 kDa. Composite anodes were obtained by directly electrospinning PEO-NFs on carbon paper, used as conductive support during the whole electrospinning process (NANON 01A equipment, from MECC Co. Ltd.). As demonstrated in our work [32], we obtained a distribution of ordered PEO-NFs on the CP surface, preferentially aligned along the conducting structures protruding from the CP surface. The protrusions act as electric field enhancers during the electrospinning process, forcing NFs to preferentially deposit on them. Moreover, CP/PEO-NFs are effective to increase the surface area to volume ratio of the electrode with respect to standard CP. All these features can enhance and facilitate proliferation of microorganisms on the surface of the composite anodes, where PEO-NFs in contact to the water base electrolyte, act as an ideal biomass carrier to promote biofilm adhesion.

2.2. MFC Architecture and Configuration

In the present work, SCMFCs were fabricated by micro milling (Al.Tip srl, Object 3D), with a squared single-chamber architecture and with an open-air cathode [35]. Membrane-less a-SCMFCs were selected, having the anodic and cathodic compartments separated by an intermediate one. In this kind of devoice, the electrolyte is in common with the anodic and cathodic compartments. The inner volume of the SCMFCs was 12.5 mL, and both anodes and cathodes showed a geometrical surface area close to 5.76 cm^2. CP/PEO-NFs were employed as composite anodes and cathode was based on CP, which had a polytetrafluoroethylene (PTFE) layer to ensure oxygen diffusion as the outer side, and an inner side where Pt/C-based catalyst was deposited, as described in our previous work [35]. Electrons were collected with titanium wires (Goodfellow Cambridge Limited), connected by a carbon cement (Leit-C) to the electrodes. With the aim to investigate the effect of tetracycline when dissolved in different electrolytes, the present work proposes the use of electrolytes that we analyzed and optimized in our previous work [35]. The first electrolyte is a conventional water-based electrolyte containing 2 g/L of sodium acetate, and it is named ***Sodium acetate***; to simulate antibiotics residues in food, we selected honey in water as the second electrolyte. We named the second electrolyte ***Honey***. To facilitate analysis and comparison, we prepared the two electrolytes adding to deionized water a concentration of honey equal to that of sodium acetate, to regulate pH to a neutral value Phosphate Buffered Saline (PBS) was also added.

Each electrolyte under analysis was tested in duplicate. A mixed consortium from seawater natural sediment, were employed. All the devices were run in fed-batch mode, i.e. the electrolyte was

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replaced when the voltage output reached a minimal value close to 0V. A data acquisition unit (Agilent 34972A) was used to monitor the a-SCMFCs performance.

In order to investigate the effecacy of SCMFCs as bio-electrochemical sensors to detect antibiotics, a small quantity of tetracycline was added to both of electrolytes, sodium acetate and honey. A concentration of tetracycline of (3.53 ± 0.13) µg/kg, referred to the amount of honey, was added. This value of tetracycline amount resulted to be lower than the MRL defined for honey as food matrix, which is close to 10 µg/kg, defined by WHO. The experiments were run with an external load of 1 kΩ applied to each a-SCMFCs. We decided to investigate whether the a-SCMFCs were able to recover their performance after exposure to toxic antibiotic. To this purpose we analyzed the energy recovery (E_{rec}) factor [36-38], calculating it for all the electrolytes (sodium acetate and honey with and without tetracycline) according to the following formula: $E_{rec} = (\int Pdt)/Vin$. The E_{rec} is indeed an energy density (J m^{-3}) calculated as the ratio of the overall energy output estimated by$(\int Pdt)$ and the inner volume of the reactor V_{in}. Electrochemical Impedance Spectroscopy (EIS) was then carried out to define internal resistance of all a-SCMFCs. A sinusoidal wave with amplitude of 25 mV was used as the signal, with the frequency ranging between 150 and 200 mHz.

3. Results and Discussion

3.1. a-SCMFCs for tetracycline detection in solium acetate-based electrolyte

In MFC-based biosensors, the microbial metabolism is directly responsible for the conversion of the chemical signal, associated to the energy trapped in the fuel, into the output electrical signal, which is associated to the output electrical energy of MFCs. Thus, the energy conversion and signal transduction steps are intimately coupled. Methods useful to promote energy conversion as thus expected to be successful to optimize biosensing as well. For this reason, we decided to employ composed nanostructured anodes, based on CP/PEO-NFs, to improve bacterial proliferation and consequently the rapidity and accuracy of SCMFCs response as biosensors. In all experiments, a load of 1 kΩ was used to analyze and define the performance of a-SMCFCs both when electrolyte contains only sodium and honey respectively is used, and when inside the electrolyte there is a certain antibiotic content. In this way the trends of current density referring to all the tests performed in a-SMCFCs, were calculated by dividing current values for anode geometric area of 5.76 cm^2. Moreover, in order to demonstrate the possibility to employ a-SCMFCs as bio-electrochemical sensors for tetracycline detection, during the first experiment, we used a water-based electrolyte in which sodium acetate was the carbon energy source, and subsequently a low amount of antibiotic, equal to (3.53 ±0.13) µg/kg, was added. As shown in **Figure 1**, a decrease of current density of about 4 times can be observed when antibiotic traces were added to the electrolyte, demonstrating the impact of the tetracycline on the metabolic activity of microorganisms.

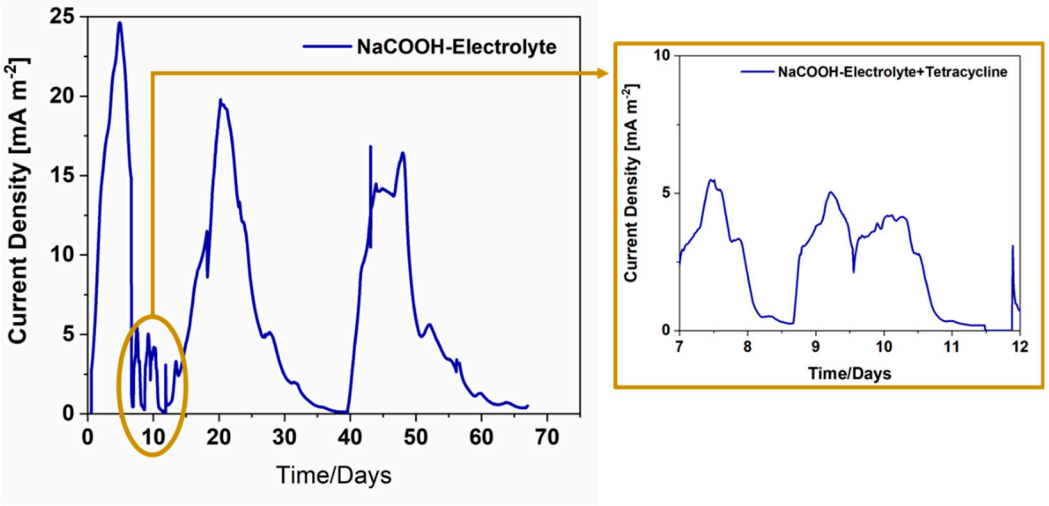

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Figure 1. Current density is produced when sodium acetate is used as electrolyte. In the yellow box the current density trend obtained when tetracycline was added to the sodium acetate electrolyte is highlighted.

Indeed, analyzing **Figure 1,** the values of current density obtained during the experiment are reported, and the first peak refers to the current density produced when only sodium acetate was present in the electrolyte. The following peaks highlight the values obtained for current density when tetracycline was added to the electrolyte. Since the intensity of current density id so reduced by the exposure to the toxicant, the peaks referring to the evet are also highlight in the yellow box. When only sodium acetate is dissolved in water-based electrolyte the maximum value of the current density was (24.8 ± 0.1) mA/m^2 [35], which is 4 times higher than the maximum value of current density of (5.87 ± 0.13) mA/m^2, obtained during the exposure to tetracycline. Moreover, it is possible to appreciate how the presence of tetracycline inside the electrolyte affects also the duration of the peaks, resulting thus shorter than when only sodium acetate was used.

3.2. a-SCMFCs for tetracycline detection in honey-based matrix

We extended the analysis investigating a-SCMFCs as bio-electrochemical sensors for tetracycline when dissolved in honey-based electrolyte. Results from these tests are fundamental to explore the possibility to use these devices for direct detection of drugs residues in food-matrices, without the necessity to extract antibiotics before their detection, thus overcoming several limitations correlated to analytical methods reported in the literature [8-16]. **Figure 2** shows overall a-SCMFCs performance, in terms of current density trends, reached when honey-based electrolyte was used and when the tetracycline was subsequently added directly in this food-matrix. Maximum current density values reached with only honey-based electrolyte, was equal to (16.29 ± 1.02) mA/m^2, which is higher one order of magnitude higher than (0.65 ± 0.16) mA/m^2, that is the current density peak referring to the presence of tetracycline into the electrolyte, as reported in **Figure 2**. An overall 95% of decrease is thus obtained for current density after exposure to tetracycline, with a reduction of the duration of the peaks. These results show a strong similarity between the behavior of the cells running on sodium acetate and those fed with honey. Thus, the comparison of Figure 1 and Figure 2 immediately clarifies that reduction of the duration of the current density peaks is independent from the specific carbon source, but is only related the presence of tetracycline that interfere with the metabolic activity of microorganisms.

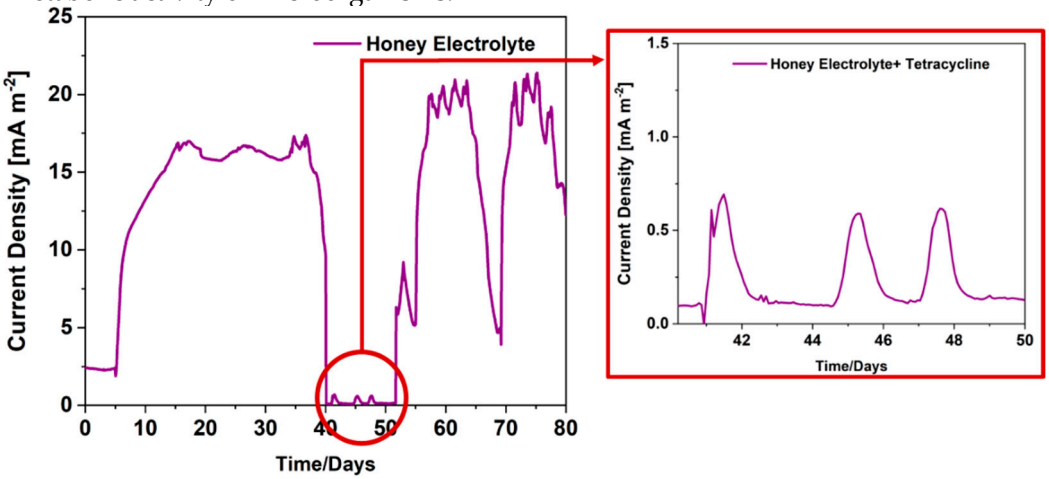

Figure 2. Current density produced when honey is used as electrolyte. In the red box the current density trend obtained when tetracycline was added to the honey-based electrolyte is highlighted.

These latter results allowed demonstrate the ability of electroactive biofilms based on mixed consortia to adapt their metabolism to use complex substrates as honey not only to produce electricity in MFC devices, but, to sense the presence of small quantities of tetracycline dissolved inside due the string impact it has of their metabolic activity.

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Finally, the comparison of **Figure 1** and **Figure 2** allows to confirm another important result, which is the capability of a-SCMFCS to recover the output current density after exposure to antibiotics for both sodium acetate-based and honey-based electrolytes. This result clearly demonstrates the robustness of the electroactive mixed consortia to survive toxic events. Indeed, the electroactive biofilm was not only able to detect a very low amount of tetracycline dissolved in water but, simultaneously, to preserve the metabolic activity despite for the contact with residues of drugs. This result shows by the stability of the maximum value of the current density associated to sodium acetate.

3.2. Energy Recovery analysis and Electrochemical Impedance Spectroscopy Results

The impact of tetracycline on the duration of current peaks can be better analysed and discussed introducing the recovered energy factor. E_{rec} is obtained by calculating the output energy produced by the MFC during an operational phase, i.e. the time between two refills of the electrolyte. This time frame is the same one during which the current density peak is observed. The values of E_{rec} calculated for the experiments previously discussed are shown in **Figure 3** for both the two electrolytes with and without tetracycline.

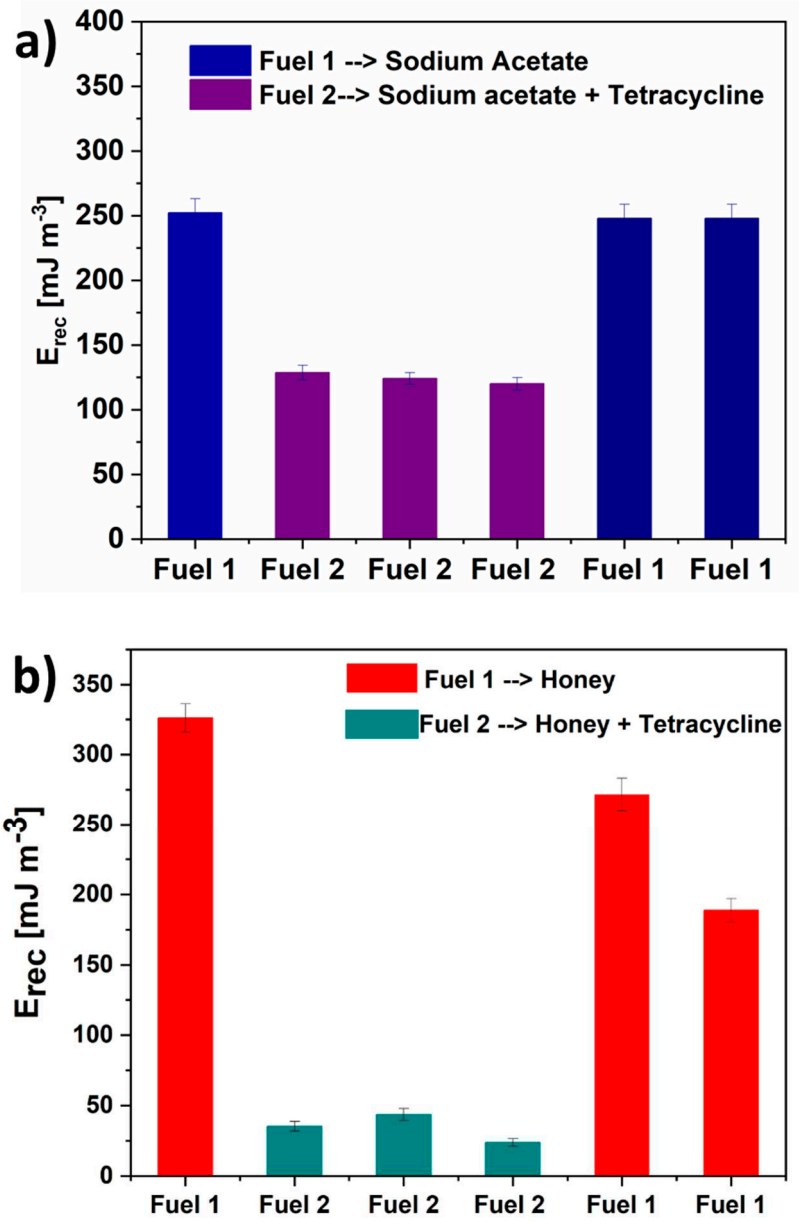

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Figure 3. (**a**) Recovered energy (E_{rec}) determined for SCMFCs using two different sodium acetate-based fuels. Fuel 1 represents the water-based electrolyte containing only sodium acetate as carbon energy source; Fuel 2 contains sodium acetate and a certain amount of tetracycline, representing thus the toxicant event for metabolic activity of microorganisms. b) Recovered energy (E_{rec}) determined for SCMFCs using two different honey- based fuels. Fuel 1 represents the honey-based electrolyte containing only honey as electrons donor; Fuel 2 contains sodium acetate and a certain amount of tetracycline, representing thus the toxicant event for metabolic activity of microorganisms.

Figure 3a) refers to the results obtained for sodium acetate-based electrolyte. It is possible to appreciate that the starting value of E_{rec} equal to (252.4 ± 10.3) mJ/m^3 was completely recovered after toxicant events, demonstrating once more the capability of microorganisms to recover their metabolic activity producing the same electrical energy output. At the same time, **Figure 3a)** confirm the decrease of the electrical performance of the devices when exposed to tetracycline, which is reflected to low value of E_{rec}, close to (134.7 ± 8.5) mJ/m^3.

A similar trend can be observed analyzing **Figure 3b)** that shows the E_{rec} values calculated honey is used as the carbon source. In this case the maximum value of E_{rec} is (326.2 ± 8.7) mJ/m^3, and refers to the output energy produced before exposure to tetracycline. Exposure to tetracycline dissolved in the food derived-matrix, cause a significant drop of the E_{rec} down to a value of (34.2 ± 4.3) mJ/m^3. E_{rec} is thus reduced of almost an order of magnitude when honey-based electrolyte contains the antibiotic, demonstrating also in this case the capability of microorganisms to act as an efficient sensitive element. Even during this experiment, the electrical energy output is recovered after the toxicant events up to a value of (262±9.8) mJ/m^3. Iyt is anyway important to observe that the recovery is not complete. Indeed, the efficiency of the catabolic activity of the biofilm to transform honey is partially reduced by the exposure to tetracycline, as demonstrated by the reduction of E_{rec}. Nevertheless, the minimum value of E_{rec} is still higher than the one reached during exposure to tetracycline of more than 6 times, and pretty close to the values obtained for devices fed with sodium acetate. These latter results confirm that a-SCMFCs can be used as bio-electrochemical sensors for the detection of antibiotics from food-derived matrices without extraction and pretreatment.

Impedance behavior was investigated by performing EIS characterizations, that allowed to provide an evaluation of internal resistance trough the analysis of the charge transfer resistance [39]. Results are proposed in **Figure 4**.

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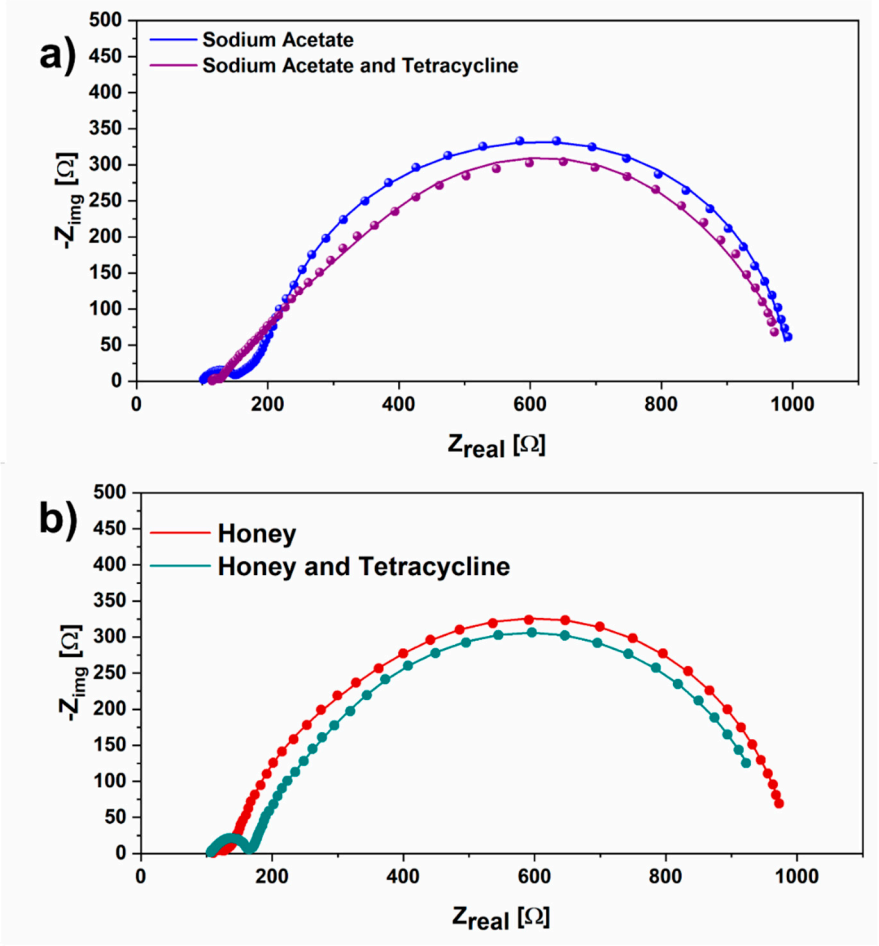

Figure 4. a) Impedance spectra of SCMFCs when sodium acetate with and without tetracycline was used as the electrolyte. Only sodium acetate (blue dots and line) and sodium acetate with tetracycline (pink dots and line). b) Impedance spectra of SCMFCs when honey with and without tetracycline was used as electrolyte. Only honey (dark red dots and line) and honey with tetracycline (dark cyan dots and line).

In particular, EIS measurements were carried on all a-SCMFCs both before and after exposure to the toxicant events. By this approach it has been possible to investigate whether the exposure to tetracycline could affect the internal resistance of a-SCMFCs after the sensing event. **Figure 4a)** represents the Nyquist plots for a-SMCFCs running on sodium acetate-based electrolyte, before and after the toxicant event. It is possible to appreciate that the total impedance values reached after the tetracycline's detection results to be close to the one obtained with only sodium acetate-based electrolyte (996 Ω and 1027 Ω, respectively). In **Figure 4b**, it can be observed that SCMFCs, initially fed with honey-based electrolyte, after exposure to the toxicant event, was characterized by total internal impedance values fully comparable with the one achieved when only honey is used as the fuel (991 Ω and 972 Ω, respectively).

These results are in line with all the other findings reported up to now and demonstrate that exposure to antibiotics has no detrimental effect on the interface between the biofilm and the electrode. Indeed, EIS measurements show that, for both the electrolytes, the charge transfer resistance at the interface is not changed by the exposure to tetracycline.

4. Conclusions

In the present work, we demonstrated that a-SCMFCs can be proposed as an effective tool for the detection of very low amount of tetracycline, close to (3.53±0.13) μg/kg, resulting to be significantly lower than the MRL defined for honey as food matrix (i.e., 10 μg/kg). Moreover, we confirmed the capability of these bio-electrochemical devices to detect antibiotic traces without the

need to perform and purification and extraction process of the drug from the matrix before the analytical analysis. All obtained results allowed confirming the pivotal role of electroactive biofilms made of microorganisms from mixed consortia, that are able not only to convert complex food matrix, such as honey, into electrical energy, but, at the same time to sense very low amount of tetracycline dissolved in the fuel, made of honey. Indeed, a current density decrease of 95% was achieved when tetracycline residues were added directly into the food-derived matrix. On the contrary, when sodium acetate was employed as fuel, the current density which is reduced of only the 25% by exposure to antibiotics, demonstrating even a higher detection ability of the a-SCMFC when detection occurs with honey used as carbon energy sources. Moreover, we evidenced that the recovered energy values in the recovery phase, i.e., after the toxicant event, achieved with sodium acetate as the carbon energy source after the close to (254±10.3) mJ/m^3 resulted to be similar to the one reached when honey is the fuel (262±9.8) mJ/m^3. At the same time, it is very important to stress that during the exposure to tetracycline an E_{rec} as low as (34.2±4.3) mJ/m^3 was obtained for devices operated with honey-based electrolyte, and this value is of one order of magnitude lower than the one reached when uncontaminated honey was employed. Finally, we proved that the electrical power output was recovered after toxicant events with both of sodium acetate and honey based electrolytes.

Author Contributions: M.Q. and G.M. conceived the work. G. M. and G.S. worked on experimental part of SCMFCs and carried out the electrochemical characterizations. M.Q and G.M. worked on the design and preparation of MFCs. C.F.P. and M.Q. organized the research activity. All authors contributed to the final manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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