Accepted Manuscript

Monitoring of Neomycin Sulfate Antibiotic in Microbial Fuel Cells

Tunc Catal, Sehnaz Yavaser, Vildan Enisoglu-Atalay, Hakan Bermek, Selma Ozilhan

PII: S0960-8524(18)31053-8
DOI: https://doi.org/10.1016/j.biortech.2018.07.122
Reference: BITE 20252

To appear in: *Bioresource Technology*

Received Date: 30 May 2018
Revised Date: 23 July 2018
Accepted Date: 24 July 2018

Please cite this article as: Catal, T., Yavaser, S., Enisoglu-Atalay, V., Bermek, H., Ozilhan, S., Monitoring of Neomycin Sulfate Antibiotic in Microbial Fuel Cells, *Bioresource Technology* (2018), doi: https://doi.org/10.1016/j.biortech.2018.07.122

Monitoring of Neomycin Sulfate Antibiotic in Microbial Fuel Cells

Tunc Catal[a,b]*, Sehnaz Yavaser[a,b], Vildan Enisoglu-Atalay[b,c], Hakan Bermek[d], Selma Ozilhan[e]

[a]Department of Molecular Biology and Genetics, [b]Istanbul Protein Research-Application and Inovation Center (PROMER), [c]Department of Bioengineering, [e]Personalized Medicine Application and Research Center (KIMER), Uskudar University 34662 Uskudar, Istanbul, Turkey

[d]Department of Molecular Biology and Genetics, Istanbul Technical University 34467-Maslak, Istanbul, Turkey

***Corresponding Author**

Phone: +90-216-4002222, Fax: +90 216 474 12 56, Email: tunc.catal@uskudar.edu.tr

ABSTRACT

Indirect detection and quantification of the neomycin sulfate antibiotic was accomplished in microbial fuel cells. Performance of the microbial fuel cells was examined on the basis of the following parameters; voltage generation, power density, current density and coulombic efficiencies. Removal of neomycin sulfate was monitored using LC-MS/MS in parallel with chemical oxygen demand and total carbohydrate removal. While neomycin sulfate was partially degraded, microbial fuel cell performance appeared to be affected and eventually inhibited by neomycin sulfate on a concentration-based fashion. In order to further examine the neomycin sulfate bio-sensing activity of the microbial fuel cell, a computational chemistry approach was used to obtain the information about the highest occupied molecular orbital-lowest unoccupied molecular orbital energy values of outer electron orbitals, their distribution, and ionization potentials (IPs). The results showed that electroactive bio-film-based MFCs can be used for sensitive detection of neomycin sulfate found in wastewaters.

Keywords: antibiotic; biosensor; electricity; microbial fuel cell; neomycin sulfate; wastewater.

1. Introduction

Quest for clean and biorenewable energy is currently at its highest level. Microbial fuel cells (MFCs) received significant attention recently for their great potential in clean energy fabrication. MFCs are electroactive biofilm-based bioreactors of miscellaneous sizes in which electrons from organic substrates are used to provide power or fuel, via the biological activity of the microorganisms (Abourached et al. 2014). While these electrons are transferred to the cathode through an external resistance where they will arrive at the terminal electron acceptor through an electrical resistance, energy is produced. MFCs thus offer wide range of

application possibilities as well as extensive potential for use in wastewater treatment, power generation for low-power applications, and bioremediation of environmental contaminants (Santoro et al. 2017).

Although the main initiative for MFC development is elimination of certain waste products and/or taking advantage of side products of certain industries to produce clean energy, one of such potential applications of MFCs is biosensing of a variety of biochemical contaminants as well as critical environmental parameters in water bodies. MFCs were thus demonstrated as perfect tools for biosensing of various compounds such as formaldehyde, copper, chromium in water quality determination (Jiang et al. 2018; Tan et al. 2018; Zhao et al. 2018; Chouler et al. 2018). More specific parameters such as toxicity, BOD monitoring, microbial activity and corrosive biofilm testing, etc. may also be investigated using MFCs as sensors (Yang et al 2015).

Antibiotics are in widespread use in human and animal health, thus, their concentration increases inevitably in the wastewaters, ground waters, and even drinking water (Tahrani et al. 2016). In independent studies published in 2006 and 2007, it was shown that twenty one and twenty eight different antibiotics were detected in a wastewater facility in Wisconsin, USA and Australia, respectively (Karthikeyan and Meyer, 2006; Watkinson et al. 2007). Even at low concentrations, these pharmaceutically-active compounds may have detrimental effects on the environment. This apparent danger to the particularly to the aquatic environment and further environmental issues drew significant attention among scientists (Lorenzo et al. 2018). Certainly, an immediate danger of this phenomenon to humans is development of novel antibiotic-resistant bacterial strains. Conventional treatment of such antibiotic-containing wastewaters is unable to accomplish the total removal of the antibiotics and requires further measures to be taken (Watkinson et al 2007).

Neomycin sulfate is one of the most commonly prescribed aminoglycosidic antibiotics. Concentrations of neomycin sulfate in pharmaceutical wastewaters reached very high levels in the recent years with the increased use of this antibiotic (Tahrani et al. 2016). Neomycin sulfate is toxic for environmental life and for human health (Aihara, 1999). Therefore, rapid and efficient online detection/quantification of neomycin is of great importance. Neomycin concentration can be estimated using traditional methods such as liquid chromatography, LC-tandem MS or immunoassays (Posyniak et al 2001; Oertel et al. 2004; Jin et al. 2006). However, these methodologies can be rather complicated and expensive, yet, sometimes not very sensitive. With the use of biosensing capability of MFCs, antibiotics can also be detected and quantified in water bodies. There is limited data in the literature on detection of antibodies using MFCs. It was recently shown that detection and quantification levofloxacin was accomplished in a MFC using $FePO_4$ nanoparticles as biocatalyst in cathode (Zeng et al. 2017). In another study, one of the currently used antibiotics, tobramycin was demonstrated to cause inhibition to MFC bacterial communities and therefore, to MFC performance (Wu et al. 2014). However, detection and quantification of a glycosidic antibiotic in MFCs was not encountered in the literature. The approach in the current study offers a cheap, efficient and highly sensitive detection and quantification method for neomycin in wastewaters for the first time. Here, chemical oxygen demand (COD) removal, total carbohydrate removal and neomycin sulfate levels were monitored to determine the influence of different concentrations of this antibiotic on MFC performance. Computational chemistry examinations by following the highest occupied molecular orbital (HOMO)-lowest unoccupied molecular orbital (LUMO) energy values, electron distributions, molecular descriptors and ionization potentials (IPs) were also conducted to better understand the antibiotic detection mechanism by the MFC and antibiotic inhibitory effect on the biofilm performance.

2. Experimental procedures

2.1. Chemicals

Neomycin sulfate [$C_{23}H_{52}N_6O_{25}S_3$, O-2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl-(1→3)-O-β-D-ribofuranosyl-(1→5)-O-[2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl-(1→4)]-2-deoxy-D-streptamine] was purchased from Calbiochem (CAS 1405-10-3, San Diego, CA, USA). D(+)-Glucose [C□H□□O□] was obtained from VWR (Alfa Aesar, Karlsruhe, Germany). All other chemicals used in the study were analytical grade and obtained from commercial sources.

2.2. MFC construction

Four single chamber mediator-less air-cathode MFCs were fabricated as described previously (Bermek et al. 2013). The MFCs consisted of the anode and cathode placed in parallel on the opposite sides of the chamber (12 mL) with a distance of 1.3 cm. Carbon cloth was used as the anode (Lot: 14032102, FuelCells, Texas, USA, 7 cm^2) and the cathode (CTO32414, FuelCells, Texas, USA, 7 cm^2) material. Cathode was coated with carbon/poly(tetrafluoroethylene) (PTFE) on the air-facing side and then with platinum (0.5 mg/cm^2 cathode area) using Nafion (CAS. No:31175, Sigma-Aldrich, USA) as binder on the internal side.

2.3. Inoculation and operation of MFCs

MFCs were inoculated with a mixed bacterial culture originally enriched from the local domestic wastewater treatment plant (Pasakoy Advanced Biological Wastewater Treatment Plant, Istanbul, Turkey).

During the acclimation period of approximately 4 days, the cells were fed with stock buffer 123 containing D-glucose (1 g/L). When ready for the experiments, they were filled with stock buffer containing D-glucose (1 g/L) as the carbon source in all the experiments with or without antibiotic addition. Each batch of MFC operation lasted for approximately 24 h, until the voltage production dropped down to 50 mV, and then terminated by emptying the liquid content for analyses. The cells were immediately refilled for the next batch with or without the antibiotic. The stock buffer-solution was prepared by dissolving the following compounds in distilled water: $NaH_2PO_4.7H_2O$ (15.47 g/L), $Na_2HPO_4.H_2O$ (5.84 g/L), NH_4Cl (0.31 g/L), KCl (0.13 g/L), a vitamin stock solution (12.5 mL/L) and a mineral stock solution (12.5 mL/L) as reported previously (Lovley and Phillip, 1988). 20 mg/L, 50 mg/L, 75 mg/L and 100 mg/L concentrations of neomycin sulfate were examined consequently in individual MFC runs with an external resistance load of 980 Ω. Neomycin sulfate was added to the MFC at a concentration of 20 mg/L tested in two consecutive MFC batch runs, then the amount of neomycin sulfate increased to the next planned concentration mentioned above. Each concentration was tested for two batch runs, including the highest concentration of 100 mg/L. All operations were carried out in a temperature controlled chamber (32±2 °C).

2.4. Analyses and calculations

A computer-aided data acquisition system (ADC24, Picolog; Cambridgeshire, UK) was hooked up to the MFCs to record the voltage generated during the operations every 11 minutes. Afterwards, solutions collected were filtered using a syringe filter (0.2 µm, VWR, Cat. no. 28145-477, USA), and then subjected to COD removal (American Public Health Association, 1992), and total carbohydrate removal analyses (anthrone method using D-glucose as the standard, Loewus et al. 1952). Removal/degradation of neomycin sulfate was investigated on the filtered samples using a liquid chromatography-tandem mass spectrometry

(LC-MS/MS) (Agilent Technologies 1200 Series 6410, Santa Clara, CA, USA) according to a previous report (Barrey and Shimelis). The inhibition ratio (I) was calculated using the following formula;

$$I\,(\%) = 100 \times (A_{M1} - A_{M2})/A_{M1}, \quad [1]$$

where A_{M1} and A_{M2} denoted the maximum current during the operation before and after neomycin sulfate addition, respectively (Wu et al. 2014). The following formula was used in the calculation of power density (mW/m^2);

$$P=IV/A, \quad [2]$$

where I is the current, V voltage, and A is the surface area of the electrode (7 cm^2). Coulombic efficiency was calculated using the following formula of;

$$CE=C_P/C_{Ti} \text{ x } 100\%, \quad [3]$$

where C_P is the total coulombs calculated by integrating the current over time and C_{Ti} is the theoretical amount of coulombs carried in the carbon source (Catal et al. 2008). All experiments were conducted twice, and standard deviations were calculated based on the mean of independent results (n=2).

2.5. Computational Methods

Neomycin sulfate molecular geometry optimization was calculated by DFT/M06-2X method with 6-31Gd basis set. The TD-SCF calculations to determine Highest Occupied Molecular Orbital (HOMO) - Lowest Unoccupied Molecular Orbital (LUMO) energies were performed at the DFT/M06-2X level of theory with 6-311++Gdp and 6-31+Gdp basis sets in gas and water phases. IEF-PCM method was used for the solvent optimization. All computational calculations were carried out through the GaussView5 (Dennington et al. 2009) molecular visualization program and Gaussian09 (Frisch et al. 2009) program package. Molecular

descriptors such as electronegativity (χ), electron affinity (EA), ionization potential (I), hardness (η), softness (S) and electrophilicity index (ω) were defined by same computational methods. The electrophilicity index (ω) shows the maximum electron flow between the donor and the acceptor atoms and is defined by the decomposition of binding energy between the atoms, and by chemical reactivity theory (Pearson, 1988). The chemical softness (S) is the measure of proclivity of a chemical substance towards alteration of its electronic configurations, thus, is in the inverse order of chemical hardness. According to Koopman's theorem, $I=-E_{HOMO}$ and $A=-E_{LUMO}$ values are derived from the frontier orbital energies of optimized neutral molecules (Ji et al. 2006). The chemical hardness and electronegativity are defined in terms of orbital energies in Eq. 4:

$$\eta \approx (E_{LUMO}-E_{HOMO})/2, \qquad x=-\mu \approx (-E_{LUMO}-E_{HOMO})/2 \qquad [4]$$

The ω and S values are calculated by the following Eq.5:

$$\omega \approx \mu^2/2\eta, \qquad S \approx 1/(2\eta) \qquad [5]$$

3. Results and discussion

Microbial fuel cells generated reproducible voltage for four consecutive batches reaching a maximum potential difference of 0.45 V with glucose, then the voltage started to decrease gradually until the medium refreshment at the end of 24 h (Fig. 1). According to MFC voltage curve, after reaching a maximum, the voltage started to decrease gradually in 24 h. Neomycin sulfate was then added in increasing concentrations during batch operations starting from 20 mg/L up to 100 mg/L in the presence of 1 g/L glucose as the carbon source. Electricity generation decreased gradually in response to the increasing concentrations of neomycin sulfate. In the presence of 100 mg/L of neomycin sulfate, potential difference decreased to

0.29 V. When the voltage levels were decreased below 0.3 V, the medium was removed and refreshed with medium without neomycin sulfate. However, at this point, voltage could not be fully recovered, holding around 0.3 V during consecutive batch operations, pointing at the inhibition of certain types of bacteria in the biofilm. Interestingly, a second voltage peak was observed during batch incubation when 50 mg/L or higher concentrations of neomycin sulfate were tested (Fig. 1). During the following operations without the antibiotic, these secondary peaks disappeared on 3rd, 4th and 5th batch operations. It could be postulated that in the presence of neomycin sulfate, a biochemical conversion of neomycin sulfate caused formation of a novel catabolic product that could participate in or used as a substrate in electricity generation. In another possible scenario, the glucose consumption profile might have changed because of antibiotic treatment promoting fermentation reactions in MFCs. This phenomenon should be further examined to better understand the influence of antibiotics on special metabolite formation as a function of time.

The neomycin sulfate level in the wastewater was reported as 16.4 mg/L in a previous study (Tahrani et al. 2016). This concentration is within the limits of detection in the current study. MFC performances were calculated using different concentrations of neomycin sulfate (Table 1). Maximum power density decreased to 117±0 mW/m^2 from 302±7 mW/m^2 and the current density dropped to 0.04 mA/m^2 from 0.07±0 mA/m^2 (100 mg/L vs. 0 mg/L neomycin sulfate, respectively). Total carbohydrate removal ratio also changed from 62±5 to 13±1% with increasing concentrations of the antibiotic (0 mg/L to 100 mg/L neomycin sulfate).

Meanwhile, with the addition of neomycin sulfate, a slight change was observed on COD removal ability, decreasing from 60±6% to 56±1%. Thus, the results showed that neomycin sulfate treatment led to a decrease in primary substrate utilization with a result of decreased power and current density levels while it did not influence COD removal rates significantly

(Table 1). Coulombic efficiency values were between 19±4 and 22±1% during the runs, and did not appear to be affected significantly by the antibiotic addition. It may be stated that the effect of the antibiotic was mainly on the electrogenic bacteria, and various other catabolic conversions were maintained by the remaining active bacterial species in the biofilm.

MFC performances in the presence of different concentrations of neomycin sulfate were compared in order to examine the bio-sensitivity of the MFCs against neomycin sulfate at mg/L levels. The inhibitory effect of the antibiotic on MFC performance showed a linearly increasing pattern of the inhibition ratio from 9.8% to 38.6% (with 20 mg/L and 100 mg/L neomycin sulfate, respectively). The increase in the inhibition ratio might be due to decreased substrate utilization and carbohydrate removal. As stated previously, power density levels as well as total carbohydrate removal rate decreased together, demonstrating that inhibition ratio is consistent with MFC performance (Table 1). This demonstrated that inhibition ratio results are consistent with MFC performance results. Wu et al. (2014) previously reported inhibition ratios in the presence of 1 mM (460 mg/L) - 4 mM (1870 mg/L) tobramycin concentration. Here, neomycin sulfate detection levels were in the range of 20-100 mg/L, showing that the MFC configuration in the current study is more sensitive against antibiotic concentration for neomycin sulfate. The possible reason for the increased inhibition ratio on the biofilm would be decreased substrate utilization as similarly pointed out by Wu et al. (2014). These results indicate that single chamber air cathode MFC configuration can be used for bio-monitoring and detection of the antibiotics at the mg/L level.

Removal of neomycin sulfate was analyzed using an LC-MS/MS setup. Results showed that over 88% of neomycin sulfate at the concentration of 20 mg/L were degraded after MFC operation. When neomycin concentration increased up to 75 mg/L, the degradation of the

antibiotic decreased. Previously, Miran et al. (2018) reported biodegradation of the sulfonamide antibiotic sulfamethoxazole in MFCs. In another study, Zhang et al. (2017) showed the potential of MFCs for treatment of antibiotic residue-containing wastewaters due to the high rates of chloramphenicol removal and energy recovery ability. While the reports mentioned above emphasize the degradation ability and strong resistance of the bacteria towards the tested antibiotics, the current study focuses mainly on biosensing of the neomycin sulfate. Thus, presence of the lower concentrations of neomycin sulfate in wastewaters could allow the current MFC setup to be efficiently utilized for biosensing purposes, without total inhibition of the system.

MFCs running in batch mode were capable of electricity production continuously if biofilm formation was left intact, and in the absence of neomycin sulfate. Nevertheless, addition of the antibiotic did not completely inhibit the electricity production, nor the other metabolic functions such as the use of carbon sources, as monitored for several weeks. MFC systems are long lasting and resilient, however, further studies should be conducted to better understand the long term effects of this antibiotic on the MFC operations.

The HOMO energies are directly related to the ionization/oxidation potential, while LUMO energies are directly related to the electron affinity and reduction potentials (Eschwege et al. 2011). The energy and distribution of the HOMO-LUMO orbitals energy difference between HOMO-LUMO gap orbital are an important stability for structures (Aihara et al. 1999). HOMO-LUMO and band gap values of the neomycin molecule calculated with the M06-2X method and 6-311++Gdp/6-31+Gdp basis sets were populated (Table 2). It is important to note that a molecule with a small band gap is more polarized and is designated as “soft molecule”, and *S* parameter supported these findings (in Table 2). It can be seen that a similar

distribution of HOMO-LUMO orbitals are determined in all studied methods and phases (Fig. 2). More importantly, the HOMO orbitals were mainly localized on the oxygen-containing six-membered tetrahydropyran ring connected to the cyclohexane ring, whereas the LUMO orbitals were distributed over the other tetrahydropyran ring and the tetrahydrofuran adjacent to it (Fig. 2).

It can be clearly stated that electron accepting groups lead to narrow band gap while electron donating groups widen the band gap (Berson et al. 2010; Matlaba et al. 2002). The most stable neomycin molecule with the narrow band gap (0.27913 eV) obtained by the M06-2X/6-31+Gdp method is observed in the water phase. It was shown that HOMO-LUMO band gap (ΔE) was smaller in the water phase when compared to that in the gas phase since the neomycin molecule in water phase has stronger electron donating ability. It appeared that increasing the concentration of neomycin sulfate up to 75 mg/L negatively affected the electron transfer from the substrate on the biofilm. Previously, the detection mechanism of levofloxacin antibiotic was investigated by quantum chemical calculation using dynamic balance of the biofilm death. The antibiotic interferred with the oxidation of substrate, blocking the cathodic reaction in air cathode configuration (Zeng et al. 2017). Therefore, it could be proposed that neomycin sulfate also affects the cathodic reaction leading to a gradual decrease in the voltage generation. In the current study, DFT SCF-TD energy indicating HOMO-LUMO potential in water phase, the E_{LUMO} energies are more favorable for reduction reaction (Table 2). Electronic molecular descriptors including electrophilic index, electron affinity and electronegativity parameters showed that in the solvent phase the values were increased. Higher concentrations of neomycin sulfate may result in an increase in electronic descriptors exhibiting better electron affinity, which, in turn, may also lead to decreased electron transfer from bacteria to the anode surface. These conclusions are in harmony with the MFC performance results.

4. Conclusion

Antibiotic neomycin sulfate was detected, quantified and partially degraded in single chamber air-cathode MFCs. Electricity was generated simultaneously in the presence of 20-100 mg/L of neomycin sulfate. Biosensing activity of the MFC was explained using the computational approaches, i.e., HOMO-LUMO energy calculations. Results demonstrated that electroactive biofilm-based MFCs could be used for sensitive detection of neomycin sulfate found in the wastewaters.

Appendix A. Supplementary data

E-supplementary data of this work can be found in online version of the paper.

References

[1] Abourached, C., Catal, T., Liu, H., 2014. Efficacy of single-chamber microbial fuel cells for removal of cadmium and zinc with simultaneous electricity production. Water Res. 51, 228-233.

[2] Aihara, J., 1999. Reduced HOMO–LUMO gap as an index of kinetic stability for polycyclic aromatic hydrocarbons. J. Phys. Chem. A. 103, 7487–7495.

[3] Berson, S., Cecioni, S., Billon, M., Kervella, Y., Bettignies, R., Bailly, S., Guillerez, S., 2010. Effect of carbonitrile and hexyloxy substituents on alternated copolymer of polythiophene-Performances in photovoltaic cells. Sol. Energy Mater Sol. Cells. 94, 699-708.

[4] Barrey, E., Shimelis, O. Aminoglycoside analysis in honey using molecularly imprinted polymer cleanup and LC/MS/MS detection, Reporter 33.1-Food.

(https://theanalyticalscientist.com/fileadmin/tas/issues/App%20Notes/06315-app-note-sigma-supplied.pdf)

[5] Bermek, H., Catal, T., Akan, S.S., Ulutaş, M.S., Kumru, M., Özgüven, M., Liu, H., Özçelik, B., Akarsubaşı, A.T., 2013. Olive mill wastewater treatment in microbial fuel cells. World J. Microbiol. Biotechnol. 30(4), 1177-1185.

[6] Cairns, J., Ruokolainen, L., Hultman, J., Tamminen, M., Virta, M., Hiltunen, T., 2018. Ecology determines how low antibiotic concentration impacts community composition and horizontal transfer of resistance genes. Commun. Biol. 1, 35.

[7] Catal, T., Xu, S., Li, K., Bermek, H., Liu, H., 2008. Electricity generation from polyalcohols in single-chamber microbial fuel cells. Biosens. Bioelectron. 24(4), 849-854.

[8] Cheng, S., Liu, H., Logan, B.E., 2006. Power densities using different cathode catalysts (Pt and CoTMPP) and polymer binders (Nafion and PTFE) in single chamber microbial fuel cells. Environ. Sci. Technol. 40, 364-369.

[9] Chouler, J., Cruz-Izquierdo, Á., Rengaraj, S., Scott, J.L., Di Lorenzo, M., 2018. A screen-printed paper microbial fuel cell biosensor for detection of toxic compounds in water. Biosens. Bioelectron. 102, 49-56.

[10] Dennington, R., Keith, T., Millam, J., 2009. Semichem Inc., GaussView, Version 5. Semichem Inc., Shawnee Mission KS.

[11] Frisch, A., Dennington, I.I.R., Keith, T., Millam, J., Nielsen, A.B., Holder, A.J., Hiscocks, J. Reference, Version 4.0, Gaussian Inc., Pittsburgh, 2007., Frisch, M.J., 2009. Gaussian 09 revision A.1. Gaussian Inc., Wallingford, CT.

[12] Ji, L., Zeng, Q.X., Wei, M.L., Jin, L.W., Zhong, Q.Y., 2006. Koopmans theorem for large molecular systems within density functional theory. J. Physical Chem. 110(43), 12005-12009.

[13] Jin, Y., Jang, J.W., Lee, M.H., Han, C.H., 2006. Development of ELISA and immunochromatographic assay for the detection of neomycin. Clin. Chim. Acta. 364(1-2), 260-6.

[14] Jiang, Y., Liang, P., Huang, X., Ren, Z.J., 2018. A novel microbial fuel cell sensor with a gas diffusion biocathode sensing element for water and air quality monitoring. Chemosphere 203, 21-25.

[15] Eschwege, K.G., Conradie, J., 2011. Redox potentials of ligands and complexes –a DFT approach. S. Afr. J. Chem. 64, 203–209.

[16] Karthikeyan, K.G., Meyer, M.T., 2006. Occurrence of antibiotics in wastewater treatment facilities in Wisconsin, USA. Sci. Total Environ. 361, 196-207.

[17] Kumru, M., Eren, H., Catal, T., Akarsubaşı, A.T., Bermek, H., 2012. Study of azo dye decolorization and determination of cathode microorganism profile in air-cathode microbial fuel cells**.** Environ. Technol. 33(16-18), 2167-2175.

[18] Loewus, F.A., 1952. Improvement in anthrone method for determination of carbohydrates. Anal. Chem. 24, 219.

[19] Lorenzo, P., Adriana, A., Jessica, S., Carles, B., Marinella, F., Marta, L., Luis, B.J., Pierre, S., 2018. Antibiotic resistance in urban and hospital wastewaters and their impact on a receiving freshwater ecosystem. Chemosphere 206, 70-82.

[20] Lovley, D.R., Phillips, E.J.P., 1988. Novel mode of microbial energy metabolism: organic carbon oxidation coupled to dissimilatory reduction of iron or manganese. Appl. Environ. Microbiol. 54, 1472–1480.

[21] Matlaba, P., Nyokong, T., 2002. Synthesis, electrochemical and photochemical properties of unsymmetrically substituted zinc phtalocyanine complexes. Polyhedron 21:2463-2472.

[22] Miran, W., Jang, J., Nawaz, M., Shahzad, A., Lee, D.S., 2018. Biodegradation of the sulfonamide antibiotic sulfamethoxazole by sulfamethoxazole acclimatized cultures in microbial fuel cells. Sci. Total Environ. 627, 1058-1065.

[23] Na, G., Lu, Z., Gao, H., Zhang, L., Li, Q., Li, R., Yang, F., Huo, C., Yao, Z. 2018. The effect of environmental factors and migration dynamics on the prevalence of antibiotic-resistant *Escherichia coli* in estuary environments. Sci. Rep. 8, article number: 1663.

[24] Oertel, R., Neumeister, V., Kirch, W. 2004. Hydrophilic interaction chromatography combined with tandem-mass spectrometry to determine six aminoglycosides in serum. J. Chromatogr. A. 1058(1–2), 197-201.

[25] Pearson, R. G., 1988. Absolute electronegativity and hardness: application to inorganic chemistry. Inorg. Chem. 27(4), 734-740.

[26] Posyniak, A., Zmudzki, J., Niedzielska, J., 2001. Sample preparation for residue determination of gentamicin and neomycin by liquid chromatography. J. Chromatogr. A. 914(1-2):59-66.

[27] Santoro, C., Arbizzani, C., Erable, B., Ieropoulos, I. 2017. Microbial fuel cells: From fundamentals to applications. A review. J. Power Sour. 356, 225-244.

[28] Tahrani, L., Van Loco, J., Ben Mansour H., Reyns T., 2016. Occurrence of antibiotics in pharmaceutical industrial wastewater, wastewater treatment plant and sea waters in Tunisia. J. Water Health 14(2), 208-13.

[29] Tan, Y.C., Kharkwal, S., Chew, K.K.W., Alwi, R., Mak, S.F.W., Ng, H.Y., 2018. Enhancing the robustness of microbial fuel cell sensor for continuous copper(II) detection against organic strength fluctuations by acetate and glucose addition. Bioresour. Technol. 259, 357-364.

[30] Wu, W., Lesnik, K.L., Xu, S., Wang, L., Liu, H., 2014. Impact of tobramycin on the performance of microbial fuel cell. Microb. Cell Fact. 13:91.

[31] Watkinson, A.J., Murby, E.J., Costanzo, S.D., 2007. Removal of antibiotics in conventional and advanced wastewater treatment: implications for environmental discharge and wastewater recycling. Water Res. 41(18), 4164-76.

[32] Yang, H., Zhou, M., Liu, M., Yang, W., Gu, T., 2015. Microbial fuel cells for biosensor applications. Biotechnol. Lett. 37(12), 2357-64.

[33] Zeng, L., Li, X., Shi, Y., Qi, Y., Huang, D., Tadé, M., Wang, S., Liu, S., 2017. FePO4 based single chamber air-cathode microbial fuel cell for online monitoring levofloxacin. Biosens. Bioelectron. 91, 367-373.

[34] Zhao, S., Liu, P., Niu, Y., Chen, Z., Khan, A., Zhang, P., Li, X., 2018. A novel early warning system based on a sediment microbial fuel cell for in situ and real time hexavalent chromium detection in industrial wastewater. Sensors (Basel) 18(2). pii: E642. doi: 10.3390/s18020642.

[35] Zhang, Q., Zhang, Y., Li, D., 2017. Cometabolic degradation of chloramphenicol via a meta-cleavage pathway in a microbial fuel cell and its microbial community. Bioresour. Technol. 229, 104-110.

TABLE AND FIGURE LEGENDS

Table 1. MFC performance using different concentrations of Neomycin (0-100 mg/L).

Table 2. The HOMO-LUMO values (eV), molecular descriptors and band gaps of neomycin molecule calculated by M062X method in gas and water phases. ΔE: Band gap; χ: electronegativity; EA: electron affinity; η: hardness; S: softness; ω: electrophilicity index.

Figure 1. Voltage production in electroactive biofilms-based MFC in the absence and presence of neomycin (20 mg/L, 50 mg/L, 75 mg/L and 100 mg/L). Arrows indicate the addition of fresh medium.

Figure 2. The calculated HOMO-LUMO energies of neomycin molecule with M06-2X/6-311++Gdp and M06-2X/6-31+Gdp basis sets in gas and water phases and bandgaps are in eV.

TABLES

Table 1.

Concentration (mg/L)	Power density (mW/m^2)	Current density (mA/cm^2)	OD_{600nm}	TC‡ removal (%)	COD* removal (%)	CE (%)
0	302±7	0.07±0	0.09±0.01	62±5	60±6	22±1
20	251±4	0.06±0	0.1±0.03	54±8	71±4	24±0
50	180±42	0.05±0	0.032±0.02	59±4	69±5	19±4
75	140±19	0.04±0	0.067±0.02	61±7	67±3	22±1
100	117±0	0.04±0	0.043±0.00	13±1	56±1	22±0

**COD: Chemical oxygen demand*
‡*TC: Total carbohydrate*
OD_{600nm}*: Optical density of the effluent solutions at 600 nm*
CE: Coulombic efficiency

Table 2.

method	HOMO	LUMO	ΔE	η	χ	ω	*S*
6-31+Gdp//gas	-0.29869	-0.00795	0.29074	-0.14537	0.15332	-0.08085	-3.43950
6-31+Gdp//water	-0.30084	-0.02171	0.27913	-0.13957	0.16128	-0.09318	-3.58256
6-311++Gdp//gas	-0.31038	-0.00555	0.30483	-0.15242	0.15797	-0.08186	-3.28052
6-311++Gdp//water	-0.31173	-0.00827	0.30346	-0.15173	0.16000	-0.08436	-3.29533

FIGURES

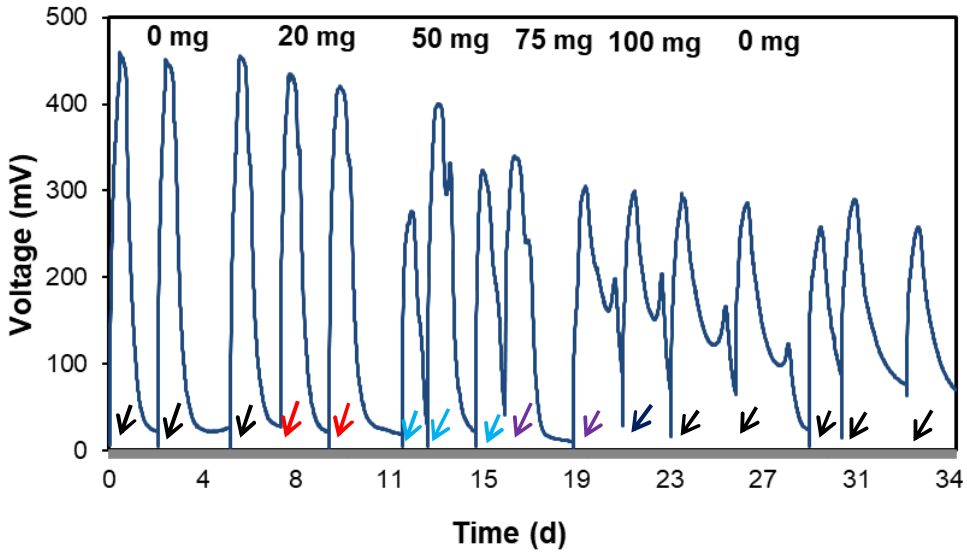

Figure 1.

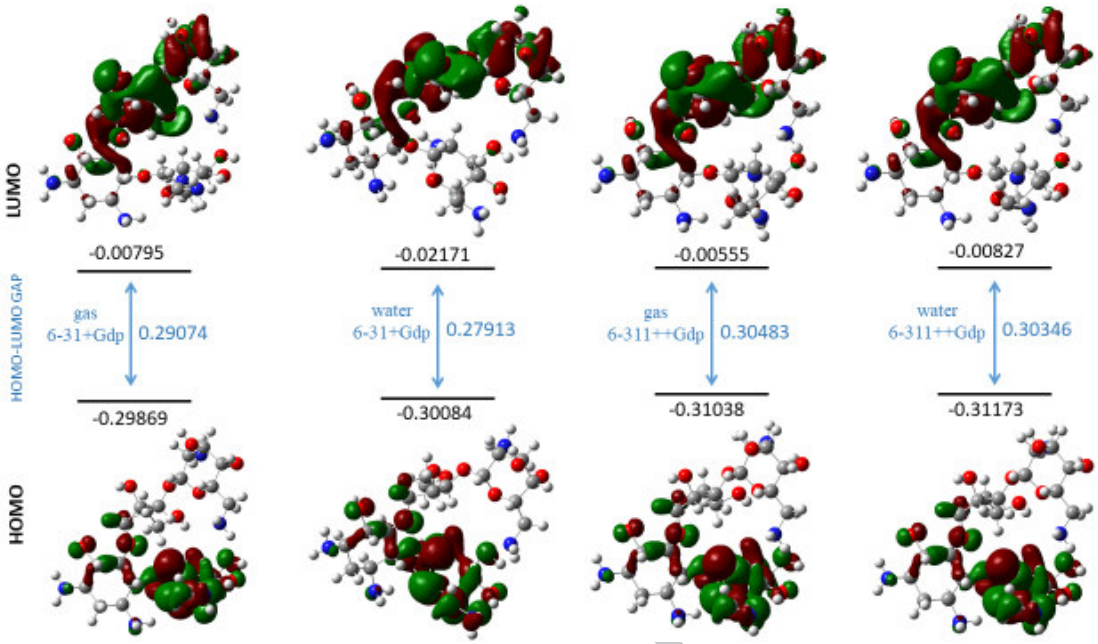

Figure 2.

Highligts

- Antibiotic neomycin sulfate was accomplished in single-chamber microbial fuel cells
- Over 88% of neomycin sulfate was removed using microbial fuel cells
- Inhibition rate increased linearly with increasing concentrations of neomycin sulfate
- Inhibition mechanism was examined using computational chemistry
- A novel approach for using microbial fuel cells as antibiotic biosensors was proposed