Lawrence Berkeley National Laboratory

Recent Work

Title

Electrodes boost microbial metabolism to mineralize antibiotics in manure.

Permalink

<https://escholarship.org/uc/item/66x2q0j7>

Authors

Rodrigo Quejigo, Jose
Tejedor-Sanz, Sara
Schroll, Reiner
et al.

Publication Date

2019-08-01

DOI

10.1016/j.bioelechem.2019.04.008

Peer reviewed

Electrodes boost microbial metabolism to mineralize antibiotics in manure

Jose Rodrigo Quejigo ^{a,b}, Sara Tejedor-Sanz ^c, Reiner Schroll ^b, Abraham Esteve-Núñez ^{a,c,*}

^a University of Alcalá, Alcalá de Henares, Madrid, Spain

^b Helmholtz Zentrum München, München, Germany

^c IMDEA-WATER Parque Tecnológico de Alcalá, Madrid, Spain

ARTICLE INFO

Article history:

Received 16 January 2019

Received in revised form 7 April 2019

Accepted 8 April 2019

Available online 17 April 2019

Keywords:

Bioremediation

Microbial fuel cell

Microbial electrochemical technologies

Sulfamethazine

Manure

Antibiotic

ABSTRACT

Livestock manures are potential sources of antibiotics in the environment. Sulfamethazine (SMZ), frequently used in veterinary medicine, can enter the environment by using manure as soil fertilizer due to its incomplete absorption in the animal gut and its unmetabolized excretion. The objective of this study was to evaluate the mineralization of ¹⁴C-labelled SMZ in manure under a new redox scenario provided by microbial electrochemical reactors, termed microbial electroremediating cells (MERC). These devices aim to overcome the electron acceptor limitation in bacterial oxidative metabolism by means of using electrodes to enhance the biodegradation of pollutants in the environment. Our results revealed that the total degradation of ¹⁴C-SMZ reached 43.5% in short term batch laboratory scale experiments under reducing conditions (−400 mV vs. Ag/AgCl). Actually, SMZ mineralization was enhanced up to 10-fold in the early stages (after 2 weeks) in comparison with an electrode-free natural attenuation assay. Moreover, mineralization showed a dependence on electrode potential, with negligible results for conditions set to +400 mV vs Ag/AgCl. The impact of merging electrodes and microorganisms for manure bioremediation suggests a promising future for this emerging technology to treat polluted livestock wastes and prevent soil and groundwater pollution.

© 2019 Published by Elsevier B.V.

1. Introduction

Antibiotics are extensively used not only in human and veterinary medicine but also as growth promoting agents in animal husbandry [1]. Due to incomplete absorption in the gut, a significant fraction of these antimicrobials are excreted unchanged or as bioactive metabolites through animal feces and urine [2–4]. Sulfamethazine (SMZ), a sulphonamide, is one of the most used antibiotics in the swine industry to treat infections and as growth promoter [1,5] and has been found in dry manure at concentrations as high as 10 mg kg^{−1} [6]. Thus, when SMZ containing manure is used as a soil fertilizer on farmland, this high mobility drug easily leaches into different environmental zones [7,8].

To date, SMZ degradation has been approached mainly using physical-chemical methods [9–11]. SMZ removal has been also reported from water and soil using biochar as an adsorption removal technique [12,13]. Moreover, a number of studies have reported biological removal in waste water reactors using activated sludge under aerobic [14] and anaerobic conditions [15]. In Both cases showed high SMZ

transformation but none of them evaluate the total degradation of the compound. Not only removal, but mineralization results for SMZ were reported under aerobic conditions by Topp et al. [16]. All these remediation strategies would not be needed by reducing the SMZ concentrations in manure before its application to agricultural fields, as a preventative bioremediation strategy. Some of the manure-handling practices to reduce manure environmental impact include storage pits and piles, composting and aerated lagoons but none of them have shown potential for SMZ degradation. Anaerobic Digestion (AD) is the most extended emerging manure management technology providing waste stabilization, organic matter digestion, odour, and pathogens reduction, a digestate, and a mixture of gases (biogas) [17]. Nevertheless the few studies to assess the potential of manure AD in SMZ removal have not reported promising results [18–20].

Consequently, new strategies for removing veterinary antibiotics in manure, especially SMZ, must be investigated. Microbial electrochemistry and microbial electrochemical reactors, where is realized, offer a set of tools for enhancing biodegradation based on its unique interaction between bacteria and electroconductive materials [21,22]. These microorganisms, denominated electroactive bacteria, oxidize organic pollutants and use an anode as an inexhaustible electron acceptor. Likewise, these microorganisms can also use electrodes as electron source for reducing pollutants [23]. They have demonstrated efficient removal of

* Corresponding author at: Department of Analytical Chemistry, Physical Chemistry and Chemical Engineering, E-28771, Alcalá de Henares, Madrid, Spain.

E-mail address: abraham.esteve@uah.es (A. Esteve-Núñez).

nutrients such as nitrates [22], chlorinated hydrocarbons [24], antibiotics [25,26], herbicides [27–29], and petroleum hydrocarbons [30,31]. Furthermore, microbial electrochemistry in combination with the anaerobic digestion of livestock manures has already been used to simultaneously generate electricity, remove organic matter and recover nitrogen [31–34], while eliminating unpleasant odors [35]. In the current work we designed and performed a bioremediation strategy using microbial electrochemical reactors, termed microbial electroremediating cells (MERC), for stimulating swine manure's native bacteria to enhance the complete biodegradation of ^{14}C -SMZ to $^{14}\text{CO}_2$.

2. Materials and methods

2.1. Chemicals

Uniformly ^{14}C ring-labelled Sulfamethazine (4-Amino-N-(4,6-dimethyl-2-pyrimidinyl) benzenesulfonamide) (^{14}C -SMZ) with a radiochemical purity of 98%, according to the producer, was purchased from Campro Scientific (AH Veenendaal, Netherlands) and used as the representative antibiotic for sulfonamides. The ^{14}C -SMZ standard had a final concentration of 0.74 mg mL^{-1} and a specific radioactivity of 2.41 kBq . Non-labelled SMZ and N₄-acetylsulfamethazine were purchased from Santa Cruz Biotechnology (Heidelberg, Germany). The $^{14}\text{CO}_2$ detected over 2% in our assays derived from the radiochemical pollutant, considering the low proportion of radiochemical impurities of the ^{14}C -SMZ applied in the series (98% purity). Scintillation cocktails (Ultima Gold XR and Ultima Flo AF) were obtained from Packard (Dreieich, Germany). All other chemicals and solvents were of analytical grade and purchased from Merck (Darmstadt, Germany).

2.2. Manure

Swine manure was obtained from a local farmer and showed no presence of SMZ. Table 1 presents the main characteristics of swine manure used during the experimental period (results by wet basis). This matrix was characterized by high organic matter content ($3.47 \pm 0.01 \text{ g L}^{-1}$, 54% biodegradable), a high C/N ratio ($12.85 \pm 0.11 \text{ w/w}$) and high conductivity ($4.2 \pm 0.3 \text{ mS cm}^{-1}$). The conductivity was measured just before the experiments with the commercially available Eca sensors UMP-1 (Umwelt-Geräte-Technik, Freising/Weihenstephan, Germany). The measurements were conducted under the same conditions (water content and temperature) as for the biodegradation assay. The pH was determined using a pH meter after mixing 2 g of milled (<2 mm) dry manure with 20 mL of 0.01 M CaCl_2 [36]. The carbon and nitrogen content was analysed using Elemental Analyser (Euro EA, Eurovector, Milano, Italy).

2.3. MERC operation

MERC were designed and operated under 3 different configurations to evaluate ^{14}C -SMZ degradation with concomitant production of

electrical current: (1) **pol-MERC** were operated at a poised anode potential of +400 mV versus Ag/AgCl reference electrode (RE-5B, BASi, United Kingdom) (+197 mV vs. SHE) by using a potentiostat (NEV2 Nanoelectra, Madrid, Spain). (2) MERC configuration with an anode potential set spontaneously by the redox potential differences across manure vertical section by connecting electrodes with a copper wire using a 5.6Ω external resistor (R), and (3) **MERC_{open}** configuration operated in open circuit with the anode and cathode disconnected. The electrodes were located, in all of the configurations at the bottom of the manure layer (anode) and above the water body (cathode) with a distance of 3 cm between each other. The electrodes used in this experiment were made of carbon felt (Sofacel, Barcelona, Spain), as this material do not show SMZ adsorption (Appendix, Fig. A1) and has adequate mechanical properties to conform the system. Additionally, it is easy to remove from the manure after the treatment, showing a low impact for the environment. The geometrical area of the electrodes was 39 cm^2 (surface area: $0.7 \text{ m}^2 \text{ g}^{-1}$). **Electrode-free controls** were assembled in the glass incubators without the presence of the electrodes and under the same water content, temperature ($30 \pm 1^\circ \text{C}$) and ^{14}C -SMZ concentrations than the other treatments.

2.4. Mineralization assays

All configurations (electrode-free control, MERC_{open}, MERC and pol-MERC) were conformed in glass incubators with 60 mL manure and equilibrated for 2 weeks (Scheme 1). After this equilibration phase ^{14}C -SMZ standard was applied to give the desired concentration of $1 (\pm 0.1) \mu\text{g mL}^{-1}$ of manure and a specific radioactivity of $2.41 \text{ kBq } \mu\text{g}^{-1}$. This point of exposure is further referred to as the initial time (t_0). The concentration was chosen to mimic concentration of SMZ found in swine manure [6]. All the experiments were incubated at $30 \pm 1^\circ \text{C}$. Water evaporation was compensated weekly by the addition of deionized water. The mineralization of ^{14}C -SMZ to $^{14}\text{CO}_2$ was studied in a closed aerated laboratory system (Scheme 2). At sampling times all the incubators were placed as part of this system and aerated for 1 h with humidified CO_2 free air (1 L h^{-1}). The evolved $^{14}\text{CO}_2$ from mineralization of ^{14}C -SMZ was adsorbed in special traps with 10 mL NaOH 1 N (Schematic 2). First sampling of the liquids in the traps was three days after application and subsequently three times per week. The $^{14}\text{CO}_2$ production rate and cumulative $^{14}\text{CO}_2$ were expressed as percentage of the applied ^{14}C -SMZ and measured during 32 days. After these 32 days all configurations were stopped but pol-MERC treatment, which it was shifted into MERC with anode and cathode connected by a 5.6Ω external resistors (R). The redox potential of the anode in MERC was set spontaneously by the redox potential differences in the

Table 1

Swine manure characteristics. The results are presented as the mean values along with standard deviations ($n = 5$). Results are expressed by wet basis.

Parameters	Swine manure
pH	7.5 ± 0.1
Conductivity (mS cm^{-1})	4.2 ± 0.3
Water content (%)	90.3
COD total (g L^{-1})	3.47 ± 0.01
COD soluble (g L^{-1})	1.87 ± 0.2
TKN (g L^{-1})	0.27 ± 0.02
NH_4^+ (g L^{-1})	0.171 ± 0.012
C / N ratio	12.85 ± 0.11
Copper (Cu) (mg kg^{-1})	1.39
Zinc (Zn) (mg kg^{-1})	5.21

Scheme 1. Overview of experimental conditions. At day 0, a pulse of SMZ was supplied to the manure. Days before inoculation (equilibration phase) are indicated as negative, days after inoculation (mineralization assays) are indicated as positive.

Scheme 2. Laboratory system for monitoring the ^{14}C -SMZ mineralization under different configurations. The zoom depicts a pol-MERC with a three-electrode system controlled for a potentiostat, polarizing the anode (working electrode) at +0.4 V vs Ag/AgCl reference electrode. 1 N NaOH solution was harvested three times per week for measuring the $^{14}\text{CO}_2$ released from ^{14}C -SMZ mineralization in manure. All systems were performed by triplicate.

manure vertical section and stayed stable at -400 mV versus Ag/AgCl. ^{14}C -SMZ mineralization was monitored until incubation day 62 to evaluate the effect of changing the redox scenario on the capability of the microbial community to mineralize ^{14}C -SMZ.

2.5. Electrochemical analysis

All electrochemical experiments were carried out under potentiostat control, using a three-electrode arrangement consisting of the working electrode, an Ag/AgCl reference electrode (+197 mV versus normal hydrogen electrode (NHE)) (RE-5B, BASi, United Kingdom) and a counter electrode. All potentials are provided versus Ag/AgCl (sat. KCl) being +0.197 V vs. standard hydrogen electrode (SHE). The anode potential in pol-MERC was continuously poised at +400 mV reference electrode using a potentiostat (NEV3 Nanoelectra, Madrid, Spain). Electrodes potential and current productions were continuously recorded with a multimeter (7700, Keithley instruments). Cyclic voltammetry (CV) was recorded before and after ^{14}C -SMZ pulses and after shifting pol-MERC into MERC to qualitatively characterize the electrochemical activity of the manure by imposing a scan rate of 1 mV s^{-1} from the open circuit voltage (OCV) potential by a potentiostat (NEV3 Nanoelectra, Madrid, Spain).

2.6. Microbial viability

Identical experiments as the ones for the mineralization assays were assembled in order to examine the influence of the antibiotic on the colonization of the anodes and on the planktonic cell viability. They were equilibrated as well for 2 weeks before SMZ application and monitored by measuring the bioelectrocatalytic oxidation current with a multimeter. Electrode and manure samples were collected at two different times of the experimental period: after 2 weeks of operation of each MERC, right before SMZ application (t_0), and after 3 weeks of SMZ

application (t_a). These samples were analysed by scanning electron microscopy (SEM) and fluorescence microscopy to study the bacterial colonization of the electrodes and the planktonic growth in the media. Samples of each carbon felt electrode were taken by cutting two small pieces of electrode with scissors. One of them was treated for SEM analysis, and the other one for cell viability analysis. All the electrode samples collected were gently rinsed with sterile phosphate buffer (50 mM) prior to its preparation in order to remove the planktonic cells. For SEM analysis, each sample of electrode was cut in half in order to examine the two faces of each electrode exposed to the media.

Samples for SEM (carbon felt samples) were fixed with 5% (v/v) glutaraldehyde in cacodylate buffer (0.2 M, pH 7.2) and dehydrated through a graded series of ethanol solutions (25, 50, 70, 90 and 100%; 10 min each stage). Subsequently, the samples were rinsed two times in acetone for 10 min and immersed in anhydrous acetone at 4°C overnight. Finally, the samples were dried in CO_2 at the critical point and coated with gold (50 nm thickness) using a Polaron E5400 coating system and argon as process gas. Micrographs were taken using a scanning electron microscope DSM-950 (Zeiss).

For fluorescence microscopy analysis, the samples were incubated in the dark at room temperature for 15 min with SYTO9 stain ($2 \mu\text{L mL}^{-1}$ of a 3.34 mM stock) and propidium iodide stain ($2 \mu\text{L mL}^{-1}$ of a 20 mM stock) from a LIVE/DEAD BacLight bacterial viability kit. Afterwards, they were washed with 30 mM phosphate buffer. In the case of the cell suspensions, the cells were harvested (10,000 rpm, 8 min) and washed twice in buffer. Images were taken in a Leica TCS-SP5, equipped with four laser (488 Ar, 561 He/Ne, 633 He/Ne and 405 UV) and 4 prism spectrophotometer detectors. The objective used was a HCX PL APO lambda blue $20.0 \times 0.70 \text{ IMM UV}$ (oil). The software was the Leica Confocal Software (LCS) for multi-dimensional image series acquisition. The software built 3D images from sequences of 32 images each one with an interval of $5 \mu\text{m}$. The sections of the electrode were obtained from the projections of each 3D image generated.

3. Results

3.1. Enhanced SMZ mineralization by using MERC

In the current section we have explored the use of different microbial electrochemical configurations (Schematic 1) to enhance the complete biodegradation of ^{14}C -SMZ to $^{14}\text{CO}_2$ in comparison with an electrode-free control. Surprisingly the native microbial community of the manure without the assistance of electrodes showed certain level (17%) of ^{14}C -SMZ mineralization after one month of incubation period (Fig. 1A). However, the presence of a negative anode potential provided in MERC (-400 mV set spontaneously by the redox potential differences across manure vertical section) enhanced by 10-fold the cumulative mineralization during the first two weeks of incubation. Furthermore, the antibiotic cumulative mineralization resulted to be 43.5% (Fig. 1A), with mineralization rates over 3% per day (Fig. 1B). In this system the two electrodes were connected by a resistor, so electrical current could flow from anode to cathode. Noteworthy, it has been previously reported that the mere presence of electroconductive material buried in soil enhanced pollutant removal by probably mediating the interspecies electron transfer among bacteria [37]. This hypothesis was explored with a MERC configuration under open circuit (MERC_{open}) was set up revealing a cumulative mineralization value of 31% in the same period of time. So electron flow from anode to cathode triggered the maximal bioremediation response, but electron flow between the conductive carbon felt and the neighbour redox environment as occurs in MERC_{open} still show a considerable bioremediation effect.

Negative potentials as low as -400 mV are typically reached in anodes buried in flooded environments due to the anoxic nature of the environment [27,29]. Although such a condition already enhanced the microbial activity, some authors have additionally reported higher bioremediation rates under a more positive anode potential by using a potentiostat as a tool for polarizing the electrode [37,28]. An electron acceptor with a higher redox potential is consistent with having a higher cell growth yield and harvesting more electrical current. This was indeed the case when polarized anodes under a pol-MERC configuration

($+400$ mV) were assayed in antibiotic-free manure (Fig. A2). However, the addition the SMZ antibiotic to the pol-MERC configuration revealed an unexpected result: a severe inhibition of the cumulative mineralization of ^{14}C -SMZ in manure so just 1.1% of the original ^{14}C was detected after 32 days of incubation (Fig. 1A). Even more intriguing, the presence of the antibiotic did not seem to affect the increasing trend for current production (Fig. A2). Furthermore pol-MERC showed a maximum current density of 15 mA m^{-2} while MERC reached only 3.5 mA m^{-2} .

In order to evaluate whether the positive electrode potential provided by pol-MERC had adverse effects on microbial SMZ degradation capacity, either by reducing the SMZ bioavailability, or damaging the microbial community, we followed the next strategy: 32 days-old pol-MERC setups were converted into MERC (referred as shifted-MERC from now on) (Schematic 1) by substituting the potentiostat equipment with a low external resistor. This new configuration led to a drop in the anode redox potential from $+400$ mV to a negative anode potential of -400 mV for the remainder of the assay. The new configuration revealed both a decrease of current, consistent with a lower redox potential, and an increase in the ^{14}C -SMZ mineralization rate. Nevertheless the total mineralization was only 9.7% after 62 days (Fig. 1B), far from the mineralization achieved in those configurations just set up as MERC from the very beginning where electrodes potentials were kept negative during the entire assay (Fig. 1A). This result supports the hypothesis that artificial condition as positive electrode potential acted as a damaging force to the SMZ-degrading microbial function.

3.2. Microbial electrochemical activity

Electron transfer between the manure microorganisms and the electrodes was analysed by cyclic voltammetry (CV) in order to understand the relationship between cumulative mineralization and bioelectrochemical activity (Fig. 2). A different electron transfer profile was observed during the adaptation period, providing inflexion peaks centred at different redox potentials under the different treatments. These differences were constant during the entire assay. Pol-MERC showed an oxidation peak centred at -0.11 and $+0.17$ V in the equilibration-phase and at -0.11 and $+0.20$ V after ^{14}C -SMZ applications (Fig. 2A and B). The peak currents of these oxidation peaks were considerably higher under the presence of the antibiotic. In contrast, the electrocatalytic wave under MERC_{open} possessed only a single inflexion point at a potential of -0.05 V and -0.01 V, before and after the antibiotic pulse respectively, both with lower current (Fig. 2A and B). Shifting from pol-MERC into MERC (shifted-MERC) seemed to change the bacteria-electrode interaction as cyclic voltammograms showed (Fig. 2C). The maximum current value of the oxidation peaks after this electrode potential shifting was considerably lower, mainly the one centred at $+0.20$ V. Furthermore, when the system was operated as a MERC for the full incubation period, the only oxidation peak detected was centred at -0.11 V but produced a higher electrical current in regards to the same peak under pol-MERC after SMZ application (Fig. 2B).

3.3. Microbial viability after SMZ application

Microbial colonization of electrodes and planktonic viability was studied by scanning electron microscopy and fluorescence microscopy (Fig. 3). In absence of antibiotic in the equilibration phase, SEM images showed a higher microbial colonization at the anodes of both pol-MERC and MERC systems in comparison with the electrodes of MERC_{open}. At this time, a larger amount of biomass was observed in between the electrode fibres at the anode of pol-MERC, which was polarized at a positive potential. Actually, this system presented a higher current production throughout the entire assay. However this microbial colonization at pol-MERC anode after SMZ application was notably reduced as revealed by SEM images. In order to better examine the electrode colonization in presence of SMZ, we stained the living cells with a fluorophore and

Fig. 1. A) Cumulative mineralization of $[^{14}\text{C}]$ -SMZ under MERC (black line-squares), electrode-free control (purple line-squares) and pol-MERC (grey line-diamond) during 32 days. B) Mineralization rate of $^{14}\text{CO}_2$ production from ^{14}C -SMZ. In pol-MERC (grey line-diamonds) the working electrode was polarized at $+0.4$ V (versus Ag/AgCl) during 32 days, when was converted into MERC (shifted-MERC) by substituting the potentiostat by a low external resistor (indicated by grey arrow) and monitored until day 62. Systems operated as MERC from the initial time (MERC) (black line-squares) showed higher mineralization rates and were operated for 32 days. Average value presented resulted from three experimental replicates. The error bars represent standard deviation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 2. Cyclic voltammetry analysis at different incubation periods for different configurations (scan rate: 1 mV s^{-1}). A. CV carried out 2 weeks after setting up the systems, during the equilibration period and before ^{14}C -SMZ addition. B. CV carried out 2 weeks after spiking the systems with ^{14}C -SMZ. C. CV carried out after pol-MERC conversion into MERC by substituting the potentiostat by a low external resistor. CVs are represented for polMERC (black line), MERC (dark blue line) and MERCocp (grey line). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

analysed the samples using confocal microscopy. The projections and the sections taken with the multi-dimensional image series acquisition allowed us to observe the scope of the microbial colonization at the carbon felt. The fluorescence images showed important differences on the biomass adhesion to the electrodes. Whereas $\text{MERC}_{\text{open}}$ and pol-MERC electrodes were barely colonized and the majority of bacteria were located at the electrode surface, MERC system showed biomass attachment to the fibres beyond $100 \mu\text{m}$ depth from the electrode surface. In the case of the $\text{MERC}_{\text{open}}$, the electrode colonization before and after SMZ application did not seem to be altered. However, the microbial presence at pol-MERC anode was visibly reduced after adding SMZ.

No visible differences were observed on the planktonic microbial density among the four different systems assayed (Fig. A3).

4. Discussion

Our experiments demonstrate how electrode-assisted bioremediation (so-called electrobioremediation) may enhance mineralization of antibiotic as ^{14}C -SMZ. Optimal mineralization happened at negative redox potentials at rates as high as 10-fold higher than electrode-free

systems at the initial stage of the assay. Interestingly just the sole presence of the electroconductive material, even under open circuit conditions ($\text{MERC}_{\text{open}}$), revealed an enhancing effect on SMZ mineralization. Thus, these results suggest that conductive material may stimulate itself the necessary redox reactions that SMZ biodegradation requires. This phenomenon may be related with direct interspecies electron transfer (DIET). This term was firstly reported by Lovley et al [38] to describe the electrochemical connection between two differing microbial species developing syntrophic metabolisms through direct contact [39]. Furthermore, Kato et al [40], also reported how magnetite can mediate interspecies electron transfer between *Geobacter sulfurreducens* and *Thiobacillus denitrificans* for stimulating acetate biodegradation. In our case the graphite may play a similar role than magnetite to stimulate SMZ biodegradation. Still, further research should be performed to have conclusive evidence about such phenomenon.

On the contrary, high anode potentials set in pol-MERC did not stimulate the microbial community to enhance SMZ degradation and erase the capacity for mineralization. Given the diprotic acid nature of SMZ, and its primarily anionic form at pH values above 7.4 (the manure pH), the bioavailability maybe one reason for the negligible mineralization. Thus, electro-adsorption processes of negatively charged SMZ may occur in contact with the positively polarized electrode ($+400 \text{ mV}$). Nevertheless, shifting from pol-MERC to MERC the electric field provided by the positive anode potential at $+400 \text{ mV}$ was erased and, consequently also the possible SMZ electro-adsorption processes on the electrode. Nevertheless the shifted-MERC configuration did not fully recover the high SMZ mineralization detected in MERC configurations. This impairment situation might be associated to an alteration on the zeta potential and bacterial membrane permeability due to the presence of a positive potential poised to the anode of pol-MERC, like other agents as cations have been previously reported to do [41,42]. Under this scenario, the antibiotic penetration through the bacterial membrane could be facilitated and damage partly the microbial community. As a consequence, the microbial capability to degrade SMZ would be minimized and cellular viability of those strains at the anode proximity could be negatively altered. Beside the mineralization data, SEM images and confocal microscopy equally support this effect, showing a minor microbial colonization at pol-MERC anodes after SMZ application, being the majority of bacteria located at the electrode surface.

The higher microbial degradation capacity of SMZ and microbial colonization at the anode in MERC contrasts with the low electrical current shown in chronoamperometric (Fig. A2) and cyclic voltammetric tests (Fig. 2), so results somehow expected due to the low redox potential of the anode. The polarized electrodes (pol-MERC) induced a higher electrochemical response in the manure microbial community. Microscopy results discard anode enrichment in pol-MERC with electroactive microbial communities due to an increase in the cell density on the electrode surface. Thus, abiotic electrochemical reactions might contribute to this higher response. The different electrochemical response is not only quantitative but the CV analysis also points to different electron transfer mechanisms (Fig. 2).

A higher concentration of planktonic dead cells was present after SMZ application regardless to electrode potential, which is consistent with the antibiotic character of this compound (Fig. A3). Nevertheless the mechanisms of the active redox couples were not significantly affected by the addition of the antibiotic (Fig. 2). The current production increased under both, MERC and pol-MERC, after the SMZ pulse (Fig. A2). This might be attributed to the fact that the electroactive community had not reached steady state within the equilibration time and/or ^{14}C -SMZ utilization as a carbon source for electroactive bacteria occurred. Indeed, increases in the electroactive microbial activity simultaneous to antibiotics removal in MERC have been previously reported [25,26]. Nevertheless SMZ ($1 (\pm 0.1) \mu\text{g m L}^{-1}$ of manure) may

Fig. 3. SEM images before and after SMZ addition, and the projections and sections observed with confocal fluorescence microscopy after SMZ application. Live cells were stained with SYTO9 stain.

contribute to the total current in a discreet range compared with the background current, based in the high organic content of the manure (3.47 g L^{-1}). Further studies are needed to completely elucidate whether direct impacts (e.g., the graphite electrode served as a direct electron acceptor for SMZ-oxidation), indirect impacts (the electrode somehow stimulated the activity of oxidizing bacteria involved in biodegradation of SMZ, for instance enforcing metabolic cooperation strategies) or both, take place under the presence of electrodes.

To the best of our knowledge, this is the first time that a significant mineralization of ^{14}C -SMZ has been reported in manure. Most of the research regarding biodegradation of SMZ has been devoted to studies in water and soil with just a few reports for the degradation of this substance within manure, where significant mineralization of SMZ has not being reported elsewhere [18–20]. Although the addition of limiting nutrients (N and P) [43] and soluble electron acceptor [44,45] have been reported for enhancing bioremediation, our results demonstrate the ability for stimulating native populations based on insoluble amendments with low impact for the environment. Our electrode-assisted treatment performs bioremediation by avoiding chemicals consumption or manure manipulation with negative environmental consequences providing an endless, low priced and sustainable terminal electron sink showing minimal environmental disturbance [46]. Furthermore, we believe that electrobioremediation shows a great potential for solving a environmental problem by minimizing antibiotic discharge into natural landscape; so our next strategy will be to integrate this electrode-assisted approach into bioelectrochemically-assisted constructed wetland (so-called METlands) for in situ pollutant degradation [47]. Such a configuration will facilitate the scaling-up to rural environments where manure and METlands may perfectly coexist.

5. Conclusions

In the present study, we demonstrate how the use of electrodes at negative anodic potential in SMZ-contaminated manure increases the

full degradation of this antibiotic. This technology can be used for in situ decontamination of antibiotic-polluted manure, as a soil preventative bioremediation strategy, with all the advantages of other in situ bioremediation approaches, while avoiding bioaugmentation or artificial addition of nutrients, which impact the environment. Thus, the results presented here enforce the establishment of electrodes as a conceivable, cost-effective, and environmentally friendly strategy for enhancing antibiotic degradation prior the pollution of a natural environment.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bioelechem.2019.04.008>.

Acknowledgment

The authors would like to thank the Alcalá University for predoctoral research grant and to the German Academic Exchange Service (DAAD) to support the work described in this dissertation. We also thank to the institute of unit Microbe-Plant Interactions (AMP)(Helmholtz Zentrum München) for supporting this research.

References

- [1] A.K. Sarmah, M.T. Meyer, A.B. Boxall, A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment, *Chemosphere* 65 (2006) 725–759.
- [2] J.C. Chee-Sanford, R.I. Mackie, S. Koike, I.G. Krapac, Y.-F. Lin, A.C. Yannarell, S. Maxwell, R.I. Aminov, Fate and transport of antibiotic residues and antibiotic resistance genes following land application of manure waste, *J. Environ. Qual.* 38 (2009) 1086–1108.
- [3] R. Wei, F. Ge, S. Huang, M. Chen, R. Wang, Occurrence of veterinary antibiotics in animal wastewater and surface water around farms in Jiangsu Province, China, *Chemosphere* 82 (2011) 1408–1414.
- [4] K.L. Nelson, V.S. Brözel, S.A. Gibson, R. Thaler, S.A. Clay, Influence of manure from pigs fed chlortetracycline as growth promotant on soil microbial community structure, *World J. Microbiol. Biotechnol.* 27 (2011) 659–668.
- [5] K.-R. Kim, G. Owens, S.-I. Kwon, K.-H. So, D.-B. Lee, Y.S. Ok, Occurrence and environmental fate of veterinary antibiotics in the terrestrial environment, *Water Air Soil Pollut.* 214 (2011) 163–174.

- [6] M.-O. Aust, F. Godlinski, G.R. Travis, X. Hao, T.A. McAllister, P. Leinweber, S. Thiele-Bruhn, Distribution of sulfamethazine, chlortetracycline and tylosin in manure and soil of Canadian feedlots after subtherapeutic use in cattle, *Environ. Pollut.* 156 (2008) 1243–1251.
- [7] M.Y. Haller, S.R. Müller, C.S. McArdell, A.C. Alder, M.J.-F. Suter, Quantification of veterinary antibiotics (sulfonamides and trimethoprim) in animal manure by liquid chromatography–mass spectrometry, *J. Chromatogr. A* 952 (2002) 111–120.
- [8] G. Hamscher, H.T. Pawelzick, H. Höper, H. Nau, Different behavior of tetracyclines and sulfonamides in sandy soils after repeated fertilization with liquid manure, *Environ. Toxicol. Chem.* 24 (2005) 861–868.
- [9] M. Cheng, Y. Liu, D. Huang, C. Lai, G. Zeng, J. Huang, Z. Liu, C. Zhang, C. Zhou, L. Qin, W. Xiong, H. Yi, Y. Yang, Prussian blue analogue derived magnetic Cu-Fe oxide as a recyclable photo-Fenton catalyst for the efficient removal of sulfamethazine at near neutral pH values, *Chem. Eng. J.* 362 (2019) 865–876.
- [10] P. Gao, M. Hao, Y. He, Y. Song, S. Yang, Effect of Fe₂O₃–ZrO₂ catalyst morphology on chlaramethazine degradation in the Fenton-like reaction, *Catalysts* 9 (2019) 85.
- [11] G. Li, K. Zhang, C. Li, R. Gao, Y. Cheng, L. Hou, Y. Wang, Solvent-free method to encapsulate polyoxometalate into metal-organic frameworks as efficient and recyclable photocatalyst for harmful sulfamethazine degrading in water, *Appl. Catal. B Environ.* 245 (2019) 753–759.
- [12] A.U. Rajapaksha, M. Vithanage, M. Ahmad, D.-C. Seo, J.-S. Cho, S.-E. Lee, S.S. Lee, Y.S. Ok, Enhanced sulfamethazine removal by steam-activated invasive plant-derived biochar, *J. Hazard. Mater.* 290 (2015) 43–50.
- [13] M. Vithanage, A.U. Rajapaksha, M. Zhang, S. Thiele-Bruhn, S.S. Lee, Y.S. Ok, Acid-activated biochar increased sulfamethazine retention in soils, *Environ. Sci. Pollut. Res.* 22 (2015) 2175–2186.
- [14] M.J. García-Galán, C.E. Rodríguez-Rodríguez, T. Vicent, G. Caminal, M.S. Díaz-Cruz, D. Barceló, Biodegradation of sulfamethazine by *Trametes versicolor*: removal from sewage sludge and identification of intermediate products by UPLC–QqTOF–MS, *Sci. Total Environ.* 409 (2011) 5505–5512.
- [15] G. Oliveira, A. Santos-Neto, M. Zaiat, Evaluation of sulfamethazine sorption and biodegradation by anaerobic granular sludge using batch experiments, *Bioprocess Biosyst. Eng.* 39 (2016) 115–124.
- [16] E. Topp, R. Chapman, M. Devers-Lamrani, A. Hartmann, R. Marti, F. Martin-Laurent, L. Sabourin, A. Scott, M. Sumarah, Accelerated biodegradation of veterinary antibiotics in agricultural soil following long-term exposure, and isolation of a sulfamethazine-degrading microbacterium sp., *J. Environ. Qual.* 42 (2013) 173–178.
- [17] J.B. Holm-Nielsen, T. Al Seadi, P. Oleskowicz-Popiel, The future of anaerobic digestion and biogas utilization, *Bioresour. Technol.* 100 (2009) 5478–5484.
- [18] S.M. Mitchell, J.L. Ullman, A.L. Teel, R.J. Watts, C. Frear, The effects of the antibiotics ampicillin, florfenicol, sulfamethazine, and tylosin on biogas production and their degradation efficiency during anaerobic digestion, *Bioresour. Technol.* 149 (2013) 244–252.
- [19] S.A. Mohring, I. Strzysch, M.R. Fernandes, T.K. Kiffmeyer, J. Tuerk, G. Hamscher, Degradation and elimination of various sulfonamides during anaerobic fermentation: a promising step on the way to sustainable pharmacy? *Environ. Sci. Technol.* 43 (2009) 2569–2574.
- [20] A. Spielmeyer, B. Breier, K. Großmeier, G. Hamscher, Elimination patterns of worldwide used sulfonamides and tetracyclines during anaerobic fermentation, *Bioresour. Technol.* 193 (2015) 307–314.
- [21] U. Schröder, F. Harnisch, L.T. Angenent, Microbial electrochemistry and technology: terminology and classification, *Energy Environ. Sci.* 8 (2015) 513–519.
- [22] S. Tejedor-Sanz, T.B. de Gregoris, J.J. Salas, L. Pastor, A. Esteve-Núñez, Integrating a microbial electrochemical system into a classical wastewater treatment configuration for removing nitrogen from low COD effluents, *Environ. Sci. Water Res. Technol.* 2 (2016) 884–893.
- [23] M. Rosenbaum, F. Aulenta, M. Villano, L.T. Angenent, Cathodes as electron donors for microbial metabolism: which extracellular electron transfer mechanisms are involved? *Bioresour. Technol.* 102 (2011) 324–333.
- [24] F. Aulenta, L. Tocca, R. Verdini, P. Reale, M. Majone, Dechlorination of trichloroethylene in a continuous-flow bioelectrochemical reactor: effect of cathode potential on rate, selectivity, and electron transfer mechanisms, *Environ. Sci. Technol.* 45 (2011) 8444–8451.
- [25] F. Harnisch, C. Gimkiewicz, B. Bogunovic, R. Kreuzig, U. Schröder, On the removal of sulfonamides using microbial bioelectrochemical systems, *Electrochem. Commun.* 26 (2013) 77–80.
- [26] W. Guo, H. Song, L. Zhou, J. Sun, Simultaneous removal of sulfanilamide and bioelectricity generation in two-chambered microbial fuel cells, *Desalin. Water Treat.* 57 (2016) 24982–24989.
- [27] J. Rodrigo Quejigo, U. Dörfler, R. Schroll, A. Esteve-Núñez, Stimulating soil microorganisms for mineralizing the herbicide isoproturon by means of microbial electroremediating cells, *Microb. Biotechnol.* 9 (2016) 369–380.
- [28] A. Domínguez-Garay, J.R. Quejigo, U. Dörfler, R. Schroll, A. Esteve-Núñez, Bioelectroventing: an electrochemical-assisted bioremediation strategy for cleaning-up atrazine-polluted soils, *Microb. Biotechnol.* 11 (1) (2017) 50–62.
- [29] A. Domínguez-Garay, K. Boltes, A. Esteve-Núñez, Cleaning-up atrazine-polluted soil by using microbial electroremediating cells, *Chemosphere* 161 (2016) 365–371.
- [30] J. Rodrigo, K. Boltes, A. Esteve-Núñez, Microbial-electrochemical bioremediation and detoxification of dibenzothiophene-polluted soil, *Chemosphere* 101 (2014) 61–65.
- [31] G. Zhang, Q. Zhao, Y. Jiao, D.-J. Lee, Long-term operation of manure-microbial fuel cell, *Bioresour. Technol.* 180 (2015) 365–369.
- [32] B. Min, J. Kim, S. Oh, J.M. Regan, B.E. Logan, Electricity generation from swine wastewater using microbial fuel cells, *Water Res.* 39 (2005) 4961–4968.
- [33] J. Ma, H. Ni, D. Su, X. Meng, Bioelectricity generation from pig farm wastewater in microbial fuel cell using carbon brush as electrode, *Int. J. Hydrog. Energy* 41 (2016) 16191–16195.
- [34] M. Cerrillo, M. Viñas, A. Bonmatí, Overcoming organic and nitrogen overload in thermophilic anaerobic digestion of pig slurry by coupling a microbial electrolysis cell, *Bioresour. Technol.* 216 (2016) 362–372.
- [35] J.R. Kim, J. Dec, M.A. Bruns, B.E. Logan, Removal of odors from swine wastewater by using microbial fuel cells, *Appl. Environ. Microbiol.* 74 (2008) 2540–2543.
- [36] E. McLean, Soil pH and Lime Requirement, *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties*, 1982 199–224.
- [37] J. Rodrigo Quejigo, A. Domínguez-Garay, U. Dörfler, R. Schroll, A. Esteve-Núñez, Anodic shifting of the microbial community profile to enhance oxidative metabolism in soil, *Soil Biol. Biochem.* 116 (2018) 131–138.
- [38] D.R. Lovley, T. Ueki, T. Zhang, N.S. Malvankar, P.M. Shrestha, K.A. Flanagan, M. Aklujkar, J.E. Butler, L. Giloteaux, A.E. Rotaru, D.E. Holmes, A.E. Franks, R. Orellana, C. Risso, K.P. Nevin, *Geobacter*: the microbe electric's physiology, ecology, and practical applications, *Adv. Microb. Physiol.* 59 (2011) 1–100.
- [39] Z.M. Summers, H.E. Fogarty, C. Leang, A.E. Franks, N.S. Malvankar, D.R. Lovley, Direct exchange of electrons within aggregates of an evolved syntrophic coculture of anaerobic bacteria, *Science* 330 (2010) 1413–1415.
- [40] S. Kato, K. Hashimoto, K. Watanabe, Microbial interspecies electron transfer via electric currents through conductive minerals, *Proc. Natl. Acad. Sci.* 109 (2012) 10042–10046.
- [41] B.E. Rittmann, P.L. McCarty, *Environmental Biotechnology: Principles and Applications*, Tata McGraw-Hill Education, 2012.
- [42] A.H. Delcour, Outer membrane permeability and antibiotic resistance, *Biochim. Biophys. Acta (BBA)-Proteins Proteomics* 1794 (2009) 808–816.
- [43] H.-Y. Yu, Y.-k. Wang, P.-c. Chen, F.-b. Li, M.-j. Chen, M. Hu, X. Ouyang, Effect of nitrate addition on reductive transformation of pentachlorophenol in paddy soil in relation to iron (III) reduction, *J. Environ. Manag.* 132 (2014) 42–48.
- [44] N. Kabelitz, J. Machackova, G. Imfeld, M. Brennerova, D.H. Pieper, H.J. Heipieper, H. Junca, Enhancement of the microbial community biomass and diversity during air sparging bioremediation of a soil highly contaminated with kerosene and BTEX, *Appl. Microbiol. Biotechnol.* 82 (2009) 565–577.
- [45] F.J.G. Frutos, O. Escolano, S. García, M. Babin, M.D. Fernández, Bioventing remediation and ecotoxicity evaluation of phenanthrene-contaminated soil, *J. Hazard. Mater.* 183 (2010) 806–813.
- [46] B.E. Logan, B. Hamelers, R. Rozendal, U. Schröder, J. Keller, S. Freguia, P. Aelterman, W. Verstraete, K. Rabaey, Microbial fuel cells: methodology and technology, *Environ. Sci. Technol.* 40 (2006) 5181–5192.
- [47] A. Aguirre-Sierra, T. Bacchetti-De Gregoris, A. Berná, J.J. Salas, C. Aragón, A. Esteve-Núñez, Microbial electrochemical systems outperform fixed-bed biofilters in cleaning up urban wastewater, *Environ. Sci. Water Res. Technol.* 2 (2016) 984–993.

Jose Rodrigo Quejigo completed his Ph.D. in microbial biotechnology at University of Alcalá (Spain) framed within the doctoral program in *Hydrology and water resources management* and entitled "Bioelectroventing: cleaning-up polluted sites using electrodes to stimulate microbial remediation activities". Currently he is working as a postdoc in the microbial bioelectrocatalysis and bioelectrotechnology group in the Helmholtz-Centre for Environmental Research GmbH – UFZ located in Leipzig, Germany.

Sara Tejedor-Sanz, Degree in Chemical Engineering at the Complutense University of Madrid and Master in Hydrology and water resources management at the University of Alcalá, Spain, where she defended her doctoral thesis (2016). The thesis was entitled "Merging microbial electrochemical systems with conventional reactor designs for treating wastewater". She is currently working as a postdoctoral researcher at the Lawrence Berkeley National Laboratory (US).

Reiner Schroll had a PhD-position at the Institute of Ecological Chemistry of the GSF-National Research Centre of Environment and Health where he finished his PhD in 1989 with a thesis about the uptake of ^{14}C -labelled chemicals from soils by plants. Since autumn 2001 to 2016 he run the working group "Interactions between organic xenobiotics and soil-plant-systems" at the Institute of Soil Ecology in the Helmholtz Zentrum München, Germany.

Abraham Esteve-Núñez, Since 2009 he is Principal investigator of the Bioe Group at the University of Alcalá (Spain). He develops his research in the field of Microbial Electrochemical Technologies (MET) applied to cleanup polluted waters and soils (www.bioelectrogenesis.com). He conducted his PhD at Spanish Science Research Council (CSIC, 2000). His postdoctoral studies were developed at the Environmental Biotechnology Center of the University of Massachusetts (USA) and at the Astrobiology Center (CSIC, Spain) to explore the physiology of electricity-producing bacteria from the genus *Geobacter*.