

Removal of *Escherichia coli* from well water using continuous laminar flow in a channel system containing PPY/Cu modified electrodes

M. B. González, L. I. Brugnioni, D. O. Flamini, L. M. Quinzani and S. B. Saidman

ABSTRACT

Polypyrrole (PPy) films modified with copper species were used for disinfection of well water contaminated with *Escherichia coli* (*E. coli*). For that purpose a laboratory-scale continuous flow system with a parallel plate flow chamber configuration was implemented operating under laminar flow. Three flow rates were considered. The testing conditions did not affect the morphology of the modified PPy films, even after 5 h of continuous use at the largest flow rate examined. The results show that the bacteria killing process can be described by a first-order kinetic law at all Reynolds numbers. As the flow rate increases, the concentration of Cu species released from the electrodes enhances, accelerating the disinfection process. Re-inoculation and Cu-recharging tests showed bactericidal effects very similar to those displayed by the freshly prepared electrodes. It is concluded that PPy/Cu-modified electrodes installed in the laboratory-scale continuous flow system are effective for the water disinfection process.

Key words | antibacterial activity, copper, laminar flow, polypyrrole, water disinfection

M. B. González (corresponding author)

D. O. Flamini

S. B. Saidman

Instituto de Ingeniería Electroquímica y Corrosión (INIEC), Departamento de Ingeniería Química, Universidad Nacional del Sur – CONICET, Av. Alem 1253, (8000) Bahía Blanca, Argentina
E-mail: belen.gonzalez@uns.edu.ar

L. I. Brugnioni

Instituto de Investigaciones Biológicas y Biomédicas del Sur (INBIOSUR), Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur, San Juan 670, (8000) Bahía Blanca, Argentina

L. M. Quinzani

Planta Piloto de Ingeniería Química (PLAPIQUI), Universidad Nacional del Sur – CONICET, Camino La Carrindanga Km 7, (8000) Bahía Blanca, Argentina

INTRODUCTION

Guaranteed access to safe water for all people is one of the largest challenges for urban sustainability. After analyzing 6,101 facilities from 54 countries, the World Health Organization (WHO) reported in 2015 that 38% of health care facilities do not have an improved water source, 19% are lacking improved sanitation and 35% do not have water and soap for hand washing (World Health Organization 2015). The availability of a secure water source, whether it is used for drinking, domestic use, food production or recreational purposes, is essential for human health. Water contaminated with pathogenic microorganisms acts as a source of infection and transmits water-borne diseases. In fact, according to a report from WHO, 842,000 deaths per year are attributable to unsafe water supply and inadequate sanitation and hygiene (World Health Organization 2014).

doi: 10.2166/wh.2018.072

Water quality monitoring can be performed by a number of indicators that may reflect its microbiological state (Ashbolt *et al.* 2001). A recommended indicator to use in water intended for drinking, sanitation or recreational reason is *Escherichia coli* (*E. coli*) because its presence suggests that there is a heightened risk of the existence of other fecal-borne bacteria and viruses (Standridge 2008; Odonkor & Ampofo 2013). *E. coli* is a Gram-negative, oxidase negative and rod shape bacterium (Croxen *et al.* 2013). This bacterium usually colonizes the gastrointestinal tract of newborns within just a few hours of birth. Although most strains of *E. coli* are harmless, there are some pathogenic ones located in contaminated water that often cause illness (Kaper *et al.* 2004).

Water disinfection is traditionally carried out using chlorine-based products. However, it has been

demonstrated that some of the by-products of this treatment are toxic and potentially carcinogenic (Pignata *et al.* 2012; Lee *et al.* 2013). In that sense, the use of surfaces modified with metals or metallic ions, such as silver and copper, stands as a promising alternative to reduce the risk of diseases caused by viruses and bacteria (Perelshtein *et al.* 2009; Dankovich & Smith 2014; Lalley *et al.* 2014). As a method for water disinfection, it is slower acting than chlorine, but is relatively safer and more effective (Yahya *et al.* 1990). Furthermore, in some circumstances it is preferable to use copper instead of silver given its lower cost and better stability. Silver ions are unstable when exposed to light and heat and they interact strongly with chloride ion in aqueous media producing AgCl precipitation (Shankar & Rhim 2014; González *et al.* 2017). Although the action mechanism of copper against microorganisms is not entirely clear, it has been proposed that the penetration of copper particles into the cell not only damage its wall but, once inside, the copper ions produce oxidative destruction, DNA degradation and may result in the death of the bacteria (Wang *et al.* 2016). The best conditions to promote the antimicrobial properties of copper species are temperatures approximately 37 °C, high humidity, high species concentration and direct contact of bacteria with copper surfaces (Vincent *et al.* 2016).

Many studies analyze the antimicrobial properties of copper in water disinfection. For example, Armstrong *et al.* (2016) investigated the inactivation kinetics of *E. coli* and *Pseudomonas aeruginosa* by copper ions in water and found reductions of 8.5 and 3.5 log₁₀ units, respectively, after 6 h of contact time. In another work, it was observed that after just 10 min chitosan nanocomposites containing copper nanoparticles not only reduce the concentration of *E. coli* in contaminated water but also those of *Staphylococcus aureus* and fungal strain *Aspergillus flavus* (Morsi *et al.* 2017).

The disinfection of water, mainly for domestic applications, can be performed using several different processes. For example, a continuous flow system is an easy and low-cost alternative. Recently, Biswas & Bandyopadhyaya (2016) considered a laboratory-scale continuous flow system operating under a laminar regime as a household application for water disinfection. They used activated carbon impregnated with silver nanoparticles that was tested against *E. coli* in 624 L of contaminated

water, and reported zero cell count after 16 days of continuous treatment. Similarly, Quang *et al.* (2012) used a filter column filled with silver nanoparticles working under laminar conditions for water disinfection. The reported results showed an *E. coli* inactivation higher than 99% with a contact time of only several seconds. Motshekga & Ray (2017) presented the inactivation of *E. coli* in river water using a continuous-flow fixed-bed column filled with chitosan-bentonite composites containing Ag and ZnO₂. According to the results, no noticeable amount of bacteria was present in the river water within the first 27 h of treatment when operating under a laminar regime.

Additionally, recent works have reported the immobilization of silver and copper species in electrosynthesized hollow rectangular-sectioned microtubes of polypyrrole (PPy) (González *et al.* 2013, 2017). The PPy/Cu-covered electrodes are relatively easy to prepare and have demonstrated a good performance in terms of corrosion protection. In addition, they display antibacterial activity towards *E. coli* in well water medium under both quiescent and hydrodynamic conditions. The possibility of applying PPy/Cu-covered films in a continuous flow system for water treatment is, then, very promising. Accordingly, in this work, PPy/Cu-modified electrodes are used for well water disinfection in a laboratory-scale continuous flow system specially designed for this purpose. The flow chamber has a parallel plate configuration operating under laminar flow. The effect of flow rate and other experimental conditions on the bacteria inactivation performance are evaluated.

MATERIAL AND METHODS

Preparation of PPy films

The electrodes consist of slices of 316 L stainless steel (316 L SS) rod (wt.% is: 17.47 Cr, 10.32 Ni, 1.88 Mn, 1.90 Mo, 0.39 Si, 0.025 C and Fe balance) of 3 mm in diameter embedded in a Teflon holder. The electrodes have 0.070 cm² of exposed area. Before proceeding to electrodeposition the PPy, the exposed surface of an electrode is mechanically abraded with SiC papers down to 1,200 grit finish, then degreased with acetone, and finally washed with triply distilled water. Following this pretreatment, the

electrodes are immediately transferred to an electrochemical cell (*Metrohm*) for PPy electro-synthesis. A large Pt sheet was used as auxiliary electrode and an Ag/AgCl (3 M KCl) electrode (*Metrohm*) as the reference. All the potentials in this work are quoted against Ag/AgCl. The electropolymerization was carried out at 25 °C in purified nitrogen atmosphere using aqueous solution containing 0.25 M pyrrole (Py) and 0.5 M sodium salicylate (NaSa). Each experiment was performed using a freshly prepared solution of Py (Sigma–Aldrich, distilled under reduced pressure). The electro-synthesis of PPy films on 316 L SS electrodes were carried out at 0.80 V for 10 min using a potentiostat–galvanostat Autolab/PGSTAT128N.

After PPy electrodeposition, the electrodes were intensively washed with distilled water. Then, copper species immobilization was carried out by dipping the PPy-covered electrode in a solution containing 0.10 M $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ + 0.10 M Na_2SO_4 of pH 1, adjusted with concentrated H_2SO_4 , for 24 h under open circuit potential (OCP) conditions.

All chemicals used were reagent grade and all solutions were prepared using triply distilled water. The morphology

of the PPy films was examined with a dual stage ISI DS 130 SEM. Atomic Absorption Spectrometer (AAS) Perkin Elmer Analyst 700 (Shelton, CT, USA) was used to determine the concentration of Cu released in well water from PPy/Cu-coated 316 L SS samples.

Flow circulation system and chamber

The bacteria-killing studies were performed on a laboratory-scale continuous flow circulation system assembled for this purpose. Figure 1 displays a schematic diagram of the system and flow chamber that contains the electrodes. The fluid is pumped through the chamber by a peristaltic pump (model PC-350, Argentine Dosibombas, Buenos Aires, Argentina) before returning to the open collecting vessel. The main pieces of the flow chamber are an insert and two plates sandwiching that insert. Together, they shape parallel plate geometry of 3 mm height (H), 30 mm width (W) and 180 mm length (L). The upper plate is planed while the bottom one has three holes aligned in the flow direction where the electrodes tightly fit. Each pair of electrodes is

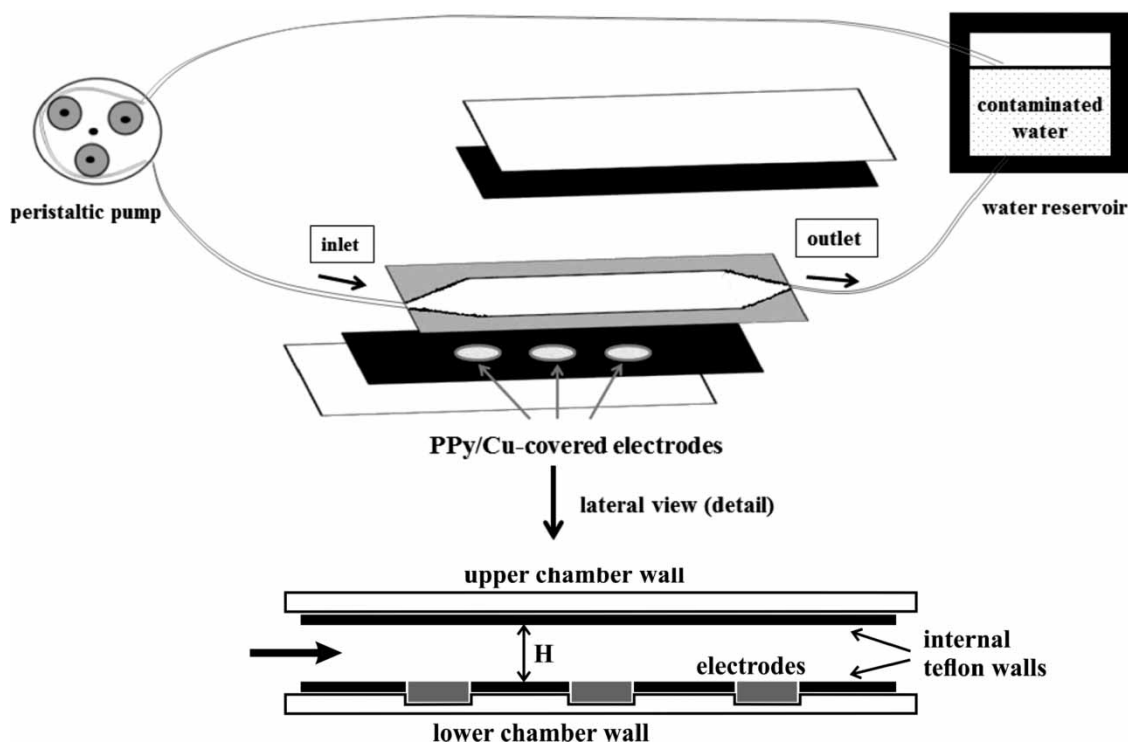


Figure 1 | Schematic representation of the flow chamber containing three PPy/Cu-covered electrodes and the continuous system including a reservoir of well water and the peristaltic pump.

separated by 15 mm (from edge to edge), the first one being located 50 mm from the flow inlet. All three pieces, the inlet and the plates, are made of Teflon and fit into an outer shell. The disinfection of the chamber and circulation system is done by recirculating 10% (v/v) bleach for 10 min at constant maximum flow rate followed by distilled water.

The parallel plate channel flow is particularly well suited for the purpose of this work. First, it is a simple well-known shear flow, and second, it may be easily scaled to handle larger flow rates, even turbulent ones. Table 1 lists the three flow rates (Q) considered in this study and the corresponding Reynolds numbers (Re) calculated as:

$$Re = \frac{\rho \langle v \rangle H}{\mu} \quad (1)$$

where ρ and μ are the density and the viscosity of the fluid, respectively, and $\langle v \rangle$ is the average fluid velocity, which corresponds to Q/WH . All three Re numbers are in the laminar flow regime. Table 1 also lists the values of the entry length for the three flow conditions, estimated as $L_e/H = 0.05 Re$. The comparison of the entry length values with the 50 mm distance from the entrance up to the first electrode and the 15 mm between electrodes, then allows assuming fully developed flow in the sections of the electrodes with velocity profiles that must be very near the parabolic one expected in developed channel flow. Besides, given the low value of the Re numbers, the thickness of the surface of the PPy/Cu-covered electrodes ($\sim 40 \mu\text{m}$) and their edge should not have a marked effect on the developed flows. Considering a parabolic velocity profile, the wall shear stress (τ_w) can be calculated for the three Re numbers. Their small values, which are also listed in Table 1, suggest that the film of PPy microtubes should not be affected by the flow.

Associated to each flow rate, a characteristic time (T) can be defined, as the fluid volume used in the experiments (100 mL) over Q . This would be the average time needed for

the tested fluid to go through the chamber once. The values of T are also listed in Table 1.

Antibacterial activity assay

The antibacterial activity of the PPy/Cu films was tested against a reference strain of *E. coli* ATCC 25922. For the experiments, a loop of frozen cells stored at -70°C in Trypticase Soy Broth (TSB) (Biokar Diagnostics, France) supplemented with 20% (v/v) glycerol (Biopack, Buenos Aires, Argentina) was grown in nutrient rich broth (Britania, Buenos Aires, Argentina) for 24 h at 37°C . Then, the cells were collected by centrifugation at $2,500 \times g$ for 10 min, washed twice with distilled sterile water and diluted with sterile well water to give a working culture of approximately 10^5 CFU (colony forming units) mL^{-1} (N_0).

The water was obtained from a well in the area of Bahía Blanca, a city located in the southwest of Buenos Aires province, Argentina ($38^\circ 43'S$ $62^\circ 16'W$). The water samples were collected in borosil glass bottles and stored at 4°C (Schulze *et al.* 2011). Table 2 lists the physicochemical properties of the water, which were obtained according to international standards (APHA 1998).

Each assay consisted of inserting 100 mL of the cell suspension in well water in the collecting vessel of the circulation system, and pumping it through the flow chamber at one of the selected flow rates for 5 h at 20°C . Every hour, an aliquot of 1 mL was taken to determine the number of cells in the water at that time (N_t). To that end, the aliquot was diluted ten-fold and the resulting dilution

Table 1 | Testing conditions

Q ($\text{m}^3 \text{s}^{-1}$)	Re	T [s]	L_e (mm)	τ_w (Pa)
0.08×10^{-6}	5	1,250	0.75	0.0018
1.0×10^{-6}	56	100	8.4	0.022
2.0×10^{-6}	115	50	17	0.044

Table 2 | Physicochemical properties of well water used in this study. Values are expressed in mg L^{-1} except for electrical conductivity ($\mu\text{S cm}^{-1}$)

Element	Values
Na	356
Mg	28.5
Ca	95.8
Total hardness	358
S (sulphate)	86.5
P (phosphate)	0.151
Cl (chloride)	408.5
pH	7.7
Electrical conductivity	1.98×10^{-3}

was plated onto plate count agar (PCA) (Britania) and incubated at 37 °C for 24 h.

The bactericidal activity (BA) was calculated as:

$$\text{BA}\% = \left(\frac{N_0 - N_t}{N_0} \right) \times 100 \quad (2)$$

Re-inoculation assay

The PPy/Cu-modified electrodes were also tested in re-inoculation assays performed at the three Re. They consisted of inoculating a fresh cell suspension (10^5 CFU mL⁻¹) in the vessel of the circulation system after 3 h of initiation of the standard procedure described above under ‘Antibacterial activity assay’. Aliquots of 1 mL were taken every hour during the following 5 hours to determine the value of N_t and the corresponding BA as a function of time.

Reusability assay

In order to evaluate the reusability of the PPy/Cu electrodes, a two-step test was performed at Re = 115. The first step consisted of a re-inoculation assay as the one described in the preceding section, but in this case the last aliquot was taken after 24 h of procedure initiation. In the second step, the electrodes were recharged by dipping them into the CuSO₄ acid solution for 24 h as described above under ‘Preparation of PPy films’, and then placed back in the flow chamber to be used in a new water disinfection test at Re = 115 (as above under ‘Antibacterial activity assay’).

RESULTS AND DISCUSSION

As discussed, the influence of the flow rate on the bacteria removal performance of PPy/Cu covered electrodes was studied using the flow chamber schematized in Figure 1. Figure 2 shows the concentration of *E. coli* as a function of time from experiments performed at the three selected Re. For comparative purposes, a test was also performed with an unmodified PPy-covered electrode at the highest flow rate. An average value of $(1.1 \pm 0.3) \times 10^5$ CFU mL⁻¹ was obtained during the 5-hour test which verifies that, as expected, no noticeable bactericidal effect occurs in the absence of an antimicrobial agent like Cu. Contrarily, the

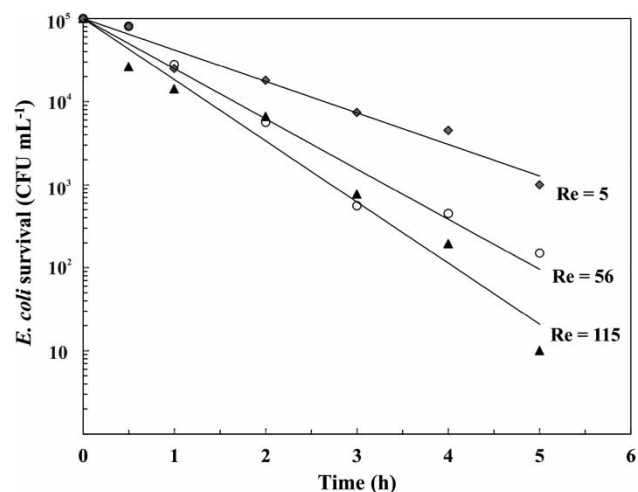


Figure 2 | Bactericidal activity for PPy/Cu-covered electrodes against *E. coli* in 100 mL of well water recirculating at different Re numbers. The full lines correspond to the exponential fitting of the experimental data. The standard deviation associated to the experimental data is no larger than 25%.

number of *E. coli* cells in well water markedly decreased with time in the case of PPy/Cu-coated electrodes under all flow conditions. Moreover, the rate of decrease augments with Re. For example, at the end of a 5 h test at Re = 115, the number of cells reduced by 10^4 CFU mL⁻¹, which corresponds to a bactericidal effect of 99.99%. In the case of Re numbers 56 and 5, the bactericidal effect after 5 h was 99.85 and 99.00%, respectively. At least three tests were performed at each condition, and the results were averaged. The standard deviation of each set of data values is no larger than 25% of the displayed mean values.

The linear behavior of the data in a semi-log representation, as in the one displayed in Figure 2, indicates that the removal processes can be described by first-order rates:

$$C_t = C_0 e^{-kt} \quad (3)$$

where C_t is the bacterial concentration in well water at time t , C_0 is the initial concentration (10^5 CFU mL⁻¹) and k is the first order rate constant, considered uniform among the bacteria population (Pal *et al.* 2006). The fitting of the C_t values to exponential functions in Figure 2 yields the values of k listed in Table 3 (coefficient of determination, R^2 , of the fittings ranges from 96 to 98%) while Figure 2 displays the resulting predictions of Equation (3). As can be seen, the value of the kinetic rate constant increases with Re and practically duplicates from the smallest to the largest Re.

Table 3 | First order rate constant (k) calculated from the exponential fitting of data in Figure 2; released concentrations of copper species in well water after 5 h of continuous flow throughout the laboratory-scale flow chamber, and kinetic rate constant calculated after re-inoculation process, all as a function of Re number

Re	k (h^{-1})	Released concentration of Cu (mg L^{-1})	k_{reino} (h^{-1})
5	0.87 ± 0.05	0.02	0.26 ± 0.08
56	1.39 ± 0.03	0.14	0.36 ± 0.07
115	1.67 ± 0.12	0.15	0.47 ± 0.09

The data in Figure 2 are displayed once again in Figure 3, but this time as a function of dimensionless time, t/T . Since T is the average time needed for 100 mL to go through the chamber one time, the ratio t/T indicates the average number of times that a fluid particle goes through the flow chamber during a test. This representation of the data shows that the number of times that the fluid has to go through the flow chamber to reach a certain level of bacterial concentration is much smaller if the Re is lesser. For example, a value of 10^3 CFU mL^{-1} is being reached after approximately 14, 120 and 210 cycles of the fluid through the chamber for Re numbers of 5, 56 and 115, respectively (taking approximately 5, 3.2 and 2.7 h, respectively).

The faster bacteria removal kinetics observed as Re increases may be explained if the release of copper from the PPy/Cu-covered electrodes augments with Re. This is in agreement with results from a previous study, where it

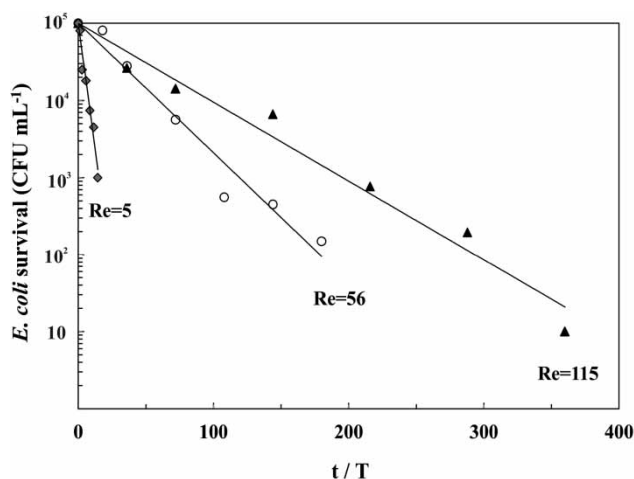


Figure 3 | *E. coli* concentration data of Figure 2 represented as a function of dimensionless time (t/T). T is the average time needed for the tested fluid to go through the chamber once. Full lines correspond to the exponential fitting of the experimental data.

was demonstrated that the transport of copper species from PPy/Cu-coated electrodes to well water and the antibacterial activity against *E. coli* increase with electrode rotation speed (González *et al.* 2017). Then, from the results above it can be proposed that the copper release from the PPy/Cu electrodes increases with flow rate and therefore the removal performance of *E. coli* becomes more effective. To confirm this conclusion, AAS analysis was carried out in order to evaluate the influence of the Re number on the amount of Cu^{2+} ions released. Table 3 includes the concentration of Cu measured in the well water after 5 h of testing at the different Re numbers. Each reported value corresponds to the average of two measurements, having a maximum dispersion of 5.7%. As can be observed, the concentration of Cu^{2+} that is detected in the well water increases with Re, being all three values below the maximum recommended level of copper in drinking water (1.3 mg L^{-1}) (United States Environmental Protection Agency 2009). The measured Cu^{2+} concentrations indicate that the smallest tested flow rate does not, in practice, affect the speed of diffusion of copper from the film to the well water (after 5 h the concentration of copper in water is still very small). However, the larger flow rates (at Re numbers of 56 and 115) benefit the release of copper from the modified electrodes, rapidly reaching similar concentrations of copper in solution (about 0.15 mg L^{-1}). The amount of copper species released was also determined in the aliquots taken after 1 and 2 h testing for Re number of 115. Values of 0.11 and 0.14 mg L^{-1} were obtained respectively. These results demonstrate that at this large Re copper ions are rapidly released, reaching a limit concentration at about 2 h.

Figure 4(a) displays a micrograph of a PPy/Cu-coated steel electrode obtained by SEM. It corresponds to a well water disinfection process performed during 5 h at $\text{Re} = 115$. The chosen image, which is representative of the whole surface, clearly shows the presence of the PPy microtubes. From the SEM analysis, two conclusions can be obtained: that there is no noticeable damage in the PPy microtubes at the end of the tests and that no evidence of bacteria can be seen on the polymer surface.

To further evaluate the laboratory-scale flow chamber performance, the PPy/Cu-modified electrodes were tested in re-inoculation assays. That is, tests were run at the three

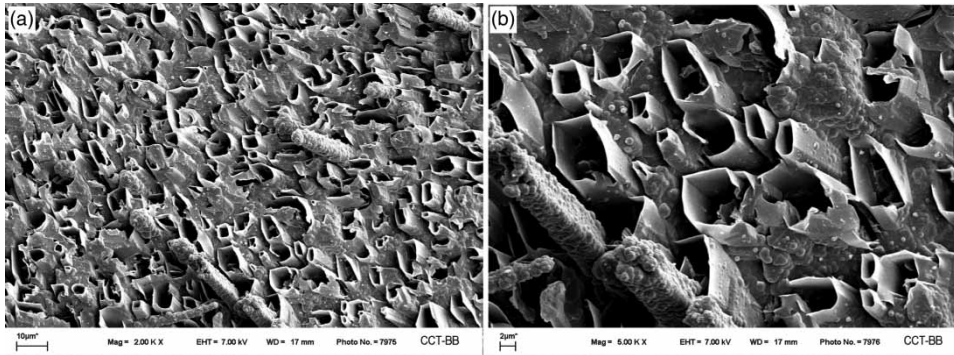


Figure 4 | SEM image of PPy/Cu-covered steel electrode after being used in a 5 h disinfection process at $Re = 115$. The PPy film was formed potentiostatically at 0.80 V during 10 min in a solution containing 0.25 M Py + 0.50 M NaSa. The copper immobilization was obtained by immersion in 0.10 M $CuSO_4$ + 0.10 M Na_2SO_4 solution of pH 1 under OCP conditions during 24 h. Magnifications: (a) 2,000 \times and (b) 5,000 \times .

Re numbers as described above under ‘Antibacterial activity assay’, but in this case an extra amount of 10^5 CFU mL $^{-1}$ of *E. coli* is re-inoculated after 3 h of procedure initiation. As an example, Figure 5 shows the concentration of bacteria as a function of time along the whole test performed at $Re = 115$. At this high Re number, the number of *E. coli* rapidly decreases with time reaching a BA of 93.4% after 3 h of testing. In the case of tests performed at Re numbers of 5 and 56, the BA determined after 3 h was 85 and 90%, respectively. After the re-inoculation process, the concentration of *E. coli* cells in well water still decreases with time under all flow conditions, but the rate of decrease is not as large as in the initial step of the test. After 8 h of testing, maximum bactericidal effects of 70, 82 and 87.5% were

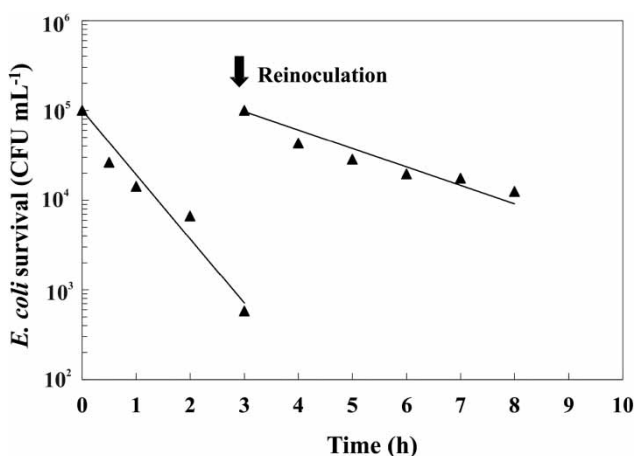


Figure 5 | Bactericidal activity of PPy/Cu-covered electrodes against *E. coli* in 100 mL of well water recirculating at $Re = 115$. An extra amount of 10^5 CFU mL $^{-1}$ of *E. coli* was added to the system after 3 h of procedure initiation (re-inoculation). The standard deviation of the displayed mean values is no larger than 20%.

obtained for increasing Re numbers. The bacteria removal kinetics still follows a first-order behavior with a rate constant value that increases with flow rate (see Table 3). Moreover, as was stated before, the value of the rate constant after re-inoculation (k_{reinoc}) at each Re number is smaller than the initial one (k). On average, the values of k_{reinoc} are 3.5 times smaller than the initial ones. This may be due to a decrease in the amount of available copper species in the PPy matrix with respect to freshly prepared PPy/Cu films.

In order to evaluate the reusability of the PPy/Cu modified electrodes, a two-step test was performed at $Re = 115$ as described above under ‘Reusability assay’. The first step was carried out over 24 h with a re-inoculation of 10^5 CFU mL $^{-1}$ after 3 h of procedure initiation. This procedure would guarantee a high depletion of copper species available for water disinfection. In the second step, the PPy/Cu modified electrodes were removed from the flow chamber and recharged by dipping them again into the $CuSO_4$ acid solution over 24 h, as described above under ‘Preparation of PPy films’. Once the electrodes were placed back in the flow chamber, a water disinfection test was restarted at $Re = 115$ using fresh well water with 10^5 CFU mL $^{-1}$ bacteria concentration. Figure 6 displays the collected results. As can be observed, the qualitative dependence between cell concentration and elapsed time determined in this stage is very similar to the one displayed in Figure 2 for the same Re number. In fact, the rate constant (k), calculated by fitting the data in Figure 6 to a first-order kinetic law, has a value of 1.54 h $^{-1}$, which is just 7% lower than the average k determined when using a freshly prepared PPy/Cu-covered electrode (1.67 ± 0.12 h $^{-1}$). In addition, statistical analysis

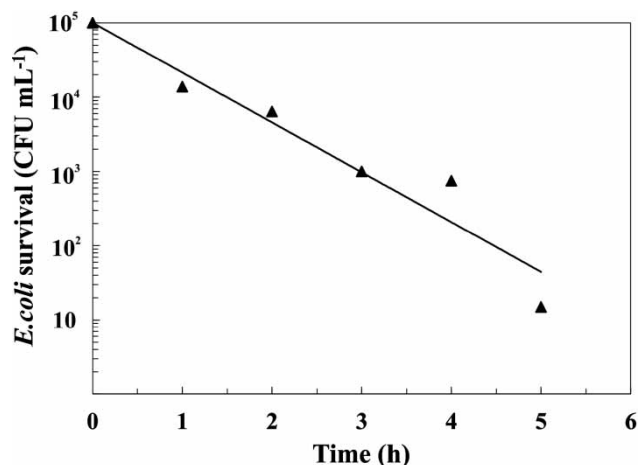


Figure 6 | Bactericidal activity of re-charged PPy/Cu modified electrodes against *E. coli* in 100 mL of well water recirculating at $Re = 115$. Full line corresponds to the exponential fitting of the experimental data.

of data demonstrated that the mean values of k do not differ among them. It can be concluded, then, that the modified electrodes can be reused if they are recharged after depletion of copper species.

DISCUSSION

A flow chamber used under laminar flow has proven to be practical for total removal of bacteria in recirculating well water without affecting the microstructure of the PPy film. Moreover, an exposed area of just 0.2 cm² located inside the chamber was sufficient to remove 10⁵ CFU mL⁻¹ of *E. coli* in 100 mL of well water after 5 to 3 h of procedure initiation when operated at Re numbers from 5 to 115. In a previous work (González *et al.* 2017) it was found that one electrode (exposed area of 0.07 cm²) was able to reduce the number of cell from 10⁵ to $\sim 7 \times 10^2$ CFU mL⁻¹ (BA = 99.3%) after 5 h of being placed in 30 mL of stagnant well water. In that sense, the present work shows that the use of a mild flow not only largely improves the removal process (BA from 99.85 to 99.99% for Re numbers of 5 to 115 in 100 mL well water) but it also does it without affecting the PPy films.

Bacteria removal under laminar flow conditions can be described with a first-order kinetic law, where the initial bacterial concentration decays exponentially with time. The augment of the value of the rate constant with flow rate

suggests that a larger flow rate improves the diffusion of Cu species to the bulk accelerating the bacteria killing process. The results suggest that, in the case of $Re = 5$, the bactericidal effect of copper may be mainly occurring in the vicinity of the PPy/Cu modified electrodes, in a process that is very effective (as shown in Figure 3) but very slow (k at $Re = 5$ has half the value than k at $Re = 115$). On the other hand, larger flow rates facilitate the removal of Cu from the film, dispersing it in the water and markedly accelerating the killing process of *E. coli*.

The non-aggressive conditions used in the implemented continuous flow system allow for the PPy-covered electrodes to be recharged with copper by simple re-immersion in acidic copper sulphate solution. The regenerated PPy/Cu films display the same bactericidal effect as the freshly prepared ones, indicating that the experimental design allows for at least a second step disinfection step.

CONCLUSIONS

A simple immersion in copper sulphate acidic solution was used as a low-cost method for cations immobilization on microstructured hollow rectangular PPy-covered 316 L SS electrode. The bacteria removal kinetics follows a first-order behavior with a rate constant value that increases with flow rate. The results demonstrated that the PPy/Cu modified electrodes are an easily rechargeable and reusable tool installable in a laboratory-scale continuous flow system for the water disinfection process.

Our findings also suggest that the use of the modified electrodes as part of a laboratory-scale continuous flow system constitutes a promising alternative for continuous water disinfection against *E. coli*. Moreover, the results presented here should propel further research in scaling up the technology to handle a larger range of flow rates in a system designed for household application.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), the Universidad Nacional del Sur (UNS), and the Agencia

Nacional de Promoción Científica y Tecnológica (ANPCyT) for the support of this project.

REFERENCES

- American Public Health Association (APHA) 1998 *Standard Methods for the Examination of Water and Wastewater*. American Water Works Association/Water Environment Federation, Washington, DC.
- Armstrong, A. M., Sobsey, M. D. & Casanova, L. M. 2016 Disinfection of *E. coli* and *P. aeruginosa* by copper in water. *J. Water Health* **14** (3), 424–432.
- Ashbolt, N. J., Grabow, W. O. K. & Snozzi, M. 2001 Indicators of microbial water quality. In: *World Health Organization. Water Quality: Guidelines, Standards and Health*, Chapter 13 (L. Fewtrell & J. Bartram, eds). IWA Publishing, London, pp. 289–316.
- Biswas, P. & Bandyopadhyaya, R. 2016 Water disinfection using silver nanoparticle impregnated activated carbon: *Escherichia coli* cell-killing in batch and continuous packed column operation over a long duration. *Water Res.* **100**, 105–115.
- Croxen, M. A., Law, R. J., Scholz, R., Keeney, K. M., Marta Wlodarska, M. & Brett, B. 2013 Recent advances in understanding enteric pathogenic *Escherichia coli*. *Clin. Microbiol. Rev.* **26**, 822–880.
- Dankovich, T. A. & Smith, J. A. 2014 Incorporation of copper nanoparticles into paper for point-of-use water purification. *Water Res.* **63**, 245–251.
- Kaper, J. B., Nataro, J. P. & Mobley, H. L. T. 2004 Pathogenic *Escherichia coli*. *Nat. Rev. Microbiol.* **2**, 123–140.
- González, M. B., Brugnoli, L. I. & Saidman, S. B. 2013 Silver deposition on polypyrrole films electrosynthesized in salicylate solutions. *Electrochim. Acta* **102**, 66–71.
- González, M. B., Brugnoli, L. I., Flamini, D. O., Quinzani, L. M. & Saidman, S. B. 2017 Immobilization and release of copper species from a microstructured polypyrrole matrix. *Environ. Monitor. Assess.* **189**, 53–64.
- Lalley, J., Dionysiou, D. D., Varma, R. S., Shankara, S., Yang, D. J. & Nadagouda, M. N. 2014 Silver-based antibacterial surfaces for drinking water disinfection. An overview. *Curr. Opin. Chem. Eng.* **3**, 25–29.
- Lee, J., Kim, E. S., Roh, B. S., Eom, S. W. & Zoh, K. D. 2013 Occurrence of disinfection by-products in tap water distribution systems and their associated health risk. *Environ. Monitor. Assess.* **185**, 7675–7691.
- Motshekga, S. C. & Ray, S. S. 2017 Highly efficient inactivation of bacteria found in drinking water using chitosan-bentonite composites: modelling and breakthrough curve analysis. *Water Res.* **111**, 213–223.
- Morsi, R. E., Alsabagh, A. M., Nasr, S. A. & Zaki, M. M. 2017 Multifunctional nanocomposites of chitosan, silver nanoparticles, copper nanoparticles and carbon nanotubes for water treatment: antimicrobial characteristics. *Int. J. Bio. Macromol.* **97**, 264–269.
- Odonkor, S. T. & Ampofo, J. K. 2013 *Escherichia coli* as an indicator of bacteriological quality of water: an overview. *Microbiol. Res.* **4**, 5–11.
- Pal, S., Joardar, J. & Song, J. M. 2006 Removal of *E. coli* from water using surface-modified activated carbon filter media and its performance over an extended use. *Environ. Sci. Tech.* **40**, 6091–6097.
- Perelshtein, I., Applerot, G., Perkas, N., Wehrschuetz-Sigl, E., Hasmann, A., Guebitz, G. & Gedanke, A. 2009 CuO-cotton nanocomposite: formation, morphology, and antibacterial activity. Cu-O cotton nanocomposites. *Surf. Coat. Tech.* **204**, 54–57.
- Pignata, C., Fea, E., Rovere, R., Degan, R., Lorenzi, E., de Ceglia, M., Schilirò, T. & Gilli, G. 2012 Chlorination in a wastewater treatment plant: acute toxicity effects of the effluent and of the recipient water body. *Environ. Monitor. Assess.* **184**, 2091–2103.
- Quang, D. V., Sarawade, P. B., Jeon, S. J., Kim, S. H., Kim, J.-K., Chai, Y. G. & Kim, H. T. 2012 Effective water disinfection using silver nanoparticle containing silica beads. *Appl. Surf. Sci.* **266**, 280–287.
- Schulze, T., Streck, G. & Paschke, A. 2011 Sampling and conservation. In: *Treatise on Water Science*, Vol. 3 (P. Wilderer & F. Frimmel, eds). Elsevier Science, The Netherlands, pp. 131–152.
- Shankar, S. & Rhim, J. W. 2014 Effect of copper salts and reducing agents on characteristics and antimicrobial activity of copper nanoparticles. *Mater. Lett.* **132**, 307–311.
- Standridge, J. 2008 *E. coli* as a public health indicator of drinking water quality. *J. Am. Water Works Assoc.* **100** (2), 65–75.
- United States Environmental Protection Agency (EPA) 2009 *National Primary Drinking Water Regulations*. Available from: www.epa.gov/ground-water-and-drinking-water/table-regulated-drinking-water-contaminants#Inorganic (accessed 06.04.2017).
- Vincent, M., Hartemann, P. & Engels-Deutsch, M. 2016 Antimicrobial application of copper. *Int. J. Hyg. Environ. Health* **219**, 585–591.
- Wang, C., Ehrhardt, C. J. & Yadavalli, V. K. 2016 Nanoscale imaging and hydrophobicity mapping of the antimicrobial effect of copper on bacterial surfaces. *Micron* **88**, 16–23.
- World Health Organization (WHO) 2014 *Water Sanitation. Health Water-Related Diseases*. Available from www.who.int/water_sanitation_health/diseases-risks/en/.
- World Health Organization (WHO) 2015 *Water, Sanitation and HYGIENE in Health Care Facilities Status in Low- and Middle-Income Countries and Way Forward*. WHO Document Production Services, Geneva, Switzerland.
- Yahya, M. T., Landeen, L. K., Messina, M. C., Kutz, S. M., Schulze, R. & Gerba, C. P. 1990 Disinfection of bacteria in water systems by using electrolytically generated copper: silver and reduced levels of free chlorine. *Can. J. Microbiol.* **36**, 109–116.