Removal of antibiotic resistant bacteria by electrolysis with diamond anodes: a pretreatment or a tertiary treatment?

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Abstract

In the present work, the influence of the water matrix on the removal of antibiotic resistant bacteria during the electro-disinfection with diamond anodes was studied, paying special attention to the disinfection efficiency and the prevention of the formation of hazardous disinfection by-products. This will allow to evaluate if electrolysis is more suitable as pretreatment of the main pollution source or as tertiary treatment of urban wastewater. To do this, electrolysis of synthetic wastewater rich in ammonium (simulating the effluent of an oxidation pond) and hospital urine intensified with three different bacteria (*E. faecalis*, *K. pneumoniae*, and *E. coli*) were carried out. Results show that the disinfection efficiency is higher in the synthetic wastewater for all the bacteria tested, but chlorate is formed as disinfection by-product. Electrogenerated hypochlorite and chloramines are the main responsible species for bacteria depletion. Presence of organics (urea, creatinine and uric acid) as additional ammonia precursors in hospital urine leads to the well-known breakpoint reaction with electrogenerated active chlorine, yielding an increasing concentration of chloramines. This helps to prevent the formation of chlorate in hospital urine because hypochlorite is mainly wasted in the oxidation of organics and the formation of chloramines. These results are of a great significance because they indicate that antibiotic resistant bacteria can be efficiently removed in complex matrixes without the formation of hazardous chlorine by-products if it is carried out as a pretreatment before discharge to WWTP.

Keywords: disinfection; antibiotic resistant bacteria; hospital effluents; electrochemical oxidation, diamond

Highlights

- Electro-disinfection with diamond anodes is suitable to destroy antibiotic resistant bacteria.
- Effluents polluted with *E. faecalis* are easier to disinfect than polluted with *K. pneumoniae* or *E. coli*.
- Higher disinfection efficiencies in urban treated wastewater, but large volume to treat.
- Occurrence of chlorate is prevented during the electrodisinfection of hospital urine.

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1. Introduction

Nowadays, the occurrence of antibiotic resistant bacteria (ARB) has become a global concern since they cause several thousands of deaths around the world every year [1-3]. Electrolysis with diamond anodes could be considered as an appropriate technology for the removal of ARB due to its efficiency in the disinfection of urban treated and surface water [4-6]. This process is based on the in-situ production of disinfectants from the oxidation of the ions naturally contained in the effluent. The main advantages of electrolysis include that the addition of chemicals is not required and the operating conditions for disinfection are usually soft [7]. These good prospects have encouraged some researchers to propose the use of diamond electrolysis as tertiary treatment after conventional wastewater treatment plants (WWTPs) [8]. Nonetheless, electrochemical processes are generally limited by mass transfer [9, 10] and by the formation of hazardous by-products [11]. These drawbacks have limited the use of this technology and have encouraged the search of different strategies to solve these problems [12, 13].

Regarding the environmental problem related to the presence of ARB in natural water, it should be pointed out that the critical source of these ARB is found in hospital facilities because many of these bacteria are excreted by urine of immunocompromised patients in whom their immune system is unable to control the infection [14]. Hospital urines are merged with wastewater from other services (laundry, kitchen…) and, finally, it is discharged to the sewerage system [15] allowing the spread of these hazardous bacteria into the environment [16, 17]. This has promoted the searching for technological solutions to decrease the impact of sanitary effluents into the environment, grouped in two main strategies: 1) boosting efficient treatments at WWTPs by the implementation of additional treatments, 2) pre-treatment before their discharge into WWTPs, that is in the pollution source. The second option is emerging as the key alternative, as it addresses the problem in more concentrated effluents and the volume of wastewater treated is further much lower than in the other case. Additionally, the treatment of urine at the point of generation could reduce the ecotoxicity risks of hospital effluents.

In this context, the goal of the present work is to evaluate the effectiveness of electrolysis with diamond anodes for the removal of ARB in two different scenarios: synthetic urban wastewater rich in ammonium (similar to that obtained in stabilization ponds, where additional disinfection is taking place by sunlight) and hospital urines. The formulation of the effluent wastewater was selected because oxidation ponds are the most similar processes, capable to explain the natural treatment of wastewater. To do this, both matrixes have been polluted with three different ARB, which have been selected as target bacteria: two bacteria commonly found in hospital urines, *Klebsiella pneumoniae* (as gram-negative ARB) and *Enterococcus faecalis* (as gram-positive ARB), and a third bacteria commonly found in urban treated wastewater and that develops antibiotic resistance, *Escherichia coli*. The disinfection tests will shed light on the role of complex matrices in the disinfection processes and, therefore, will allow to establish the best strategy to remove target bacteria and to avoid the formation of undesirable disinfection-by-products.

2. Material and methods

2.1. Microorganisms and chemicals

Bacteria strains (pure culture) used during electrodisinfection were *E. coli* ATCC 25922, *K. pneumoniae* ATCC 4352 and *E. faecalis* ATCC 19433 (CECT, Spain). Synthetic urine and wastewater were prepared according to the formulation shown in Table 1. In this work, the formulation used for synthetic wastewater simulates the characteristics of the effluent of an oxidation pond, natural systems that simulate what nature does in natural environments by itself and where the disinfection by sunlight occurs. Sodium carbonate and acetone were used for the determination of anions by ion chromatography. All chemicals were analytical grade and used as received (Sigma Aldrich, Spain). Calcium chloride dihydrate (BiMedia 001B) was used for the determination of *K. pneumoniae* and *E. faecalis* and, sodium dodecyl sulfate (BiMedia 155A) was used for the determination of *E. coli* (SY-LAB). All solutions were prepared with double deionized water (Millipore Milli-Q).

2.2. Experimental procedure

Electrochemical disinfection experiments were carried out under batch-operation mode. Boron doped diamond and Stainless Steel with a circular geometric area of 78 cm^2 were used as anode and cathode, respectively. The interelectrode gap was 9 mm. More details about the electrochemical cell have been reported elsewhere [18]. All experiments were carried out under galvanostatic conditions and the current density applied was 10 A m^{-2}. This low value has been selected considering other results reported in the literature related to the applicability of electrodisinfection process to urine and wastewater [18].

Table 1. Target effluents composition.

Species	Urban treated wastewater*	Hospital urine
Cl^- (mg dm^{-3})	148.22	475.52
NO_3^- (mg dm^{-3})	10.94	-
PO_4^{3-} (mg dm^{-3})	5.79	59.94
SO_4^{2-} (mg dm^{-3})	299.37	135.58
CO_3^{2-} (mg dm^{-3})	90.58	94.35
Na^+ (mg dm^{-3})	97.39	72.34
K^+ (mg dm^{-3})	31.47	524.45

NH_4^+ (mg dm^{-3})	40.87	22.72
Ca^{2+} (mg dm^{-3})	79.48	10.99
Mg^{2+} (mg dm^{-3})	30.63	34.33
Humic acids (mg dm^{-3})	20.00	-
CH_4N_2O (mg dm^{-3})	-	3333.33
$C_4H_7N_3O$ (mg dm^{-3})	-	166.67
$C_5H_4N_4O_3$ (mg dm^{-3})	-	50.00

*Effluent of an oxidation pond

2.5. Analytical methods

Bacteria counts were performed by an indirect impedance method using a μ-Trac® 4200 system. This technology uses a standard impedance signal that is related to the concentration of bacteria in CFU mL^{-1} through an initial correlation between μ-Trac® and plate counts. The measurement of the concentration of free and combined chlorine species has been reported elsewhere [19].

3. Results and discussion

Figure 1a shows the decay in the population of three different ARB with the applied electric charge during the electrochemical disinfection with diamond anodes of synthetic urban treated wastewater and hospital urine. All electrolysis tests were carried out at 10 A m^{-2}. The initial bacteria population used was similar in both effluents to evaluate the process efficiency in similar conditions, although a lower population may be expected in a real urban treated wastewater [20].

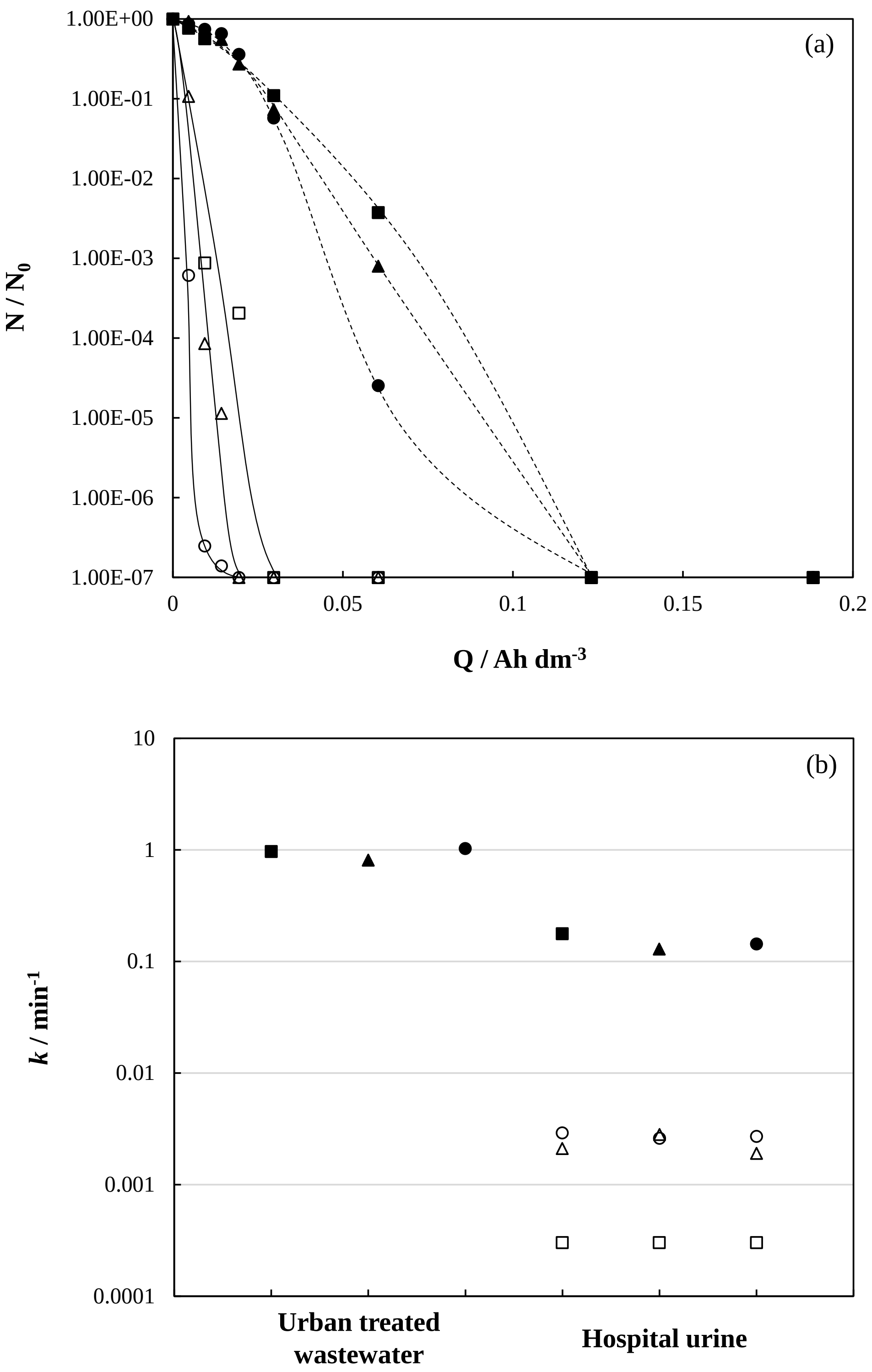

Figure 1. (a) Influence of water matrix of the removal of antibiotic resistant bacteria by electrolysis with diamond anodes. (■) *E. coli*; (▲) *K. pneumoniae*; (●) *E. faecalis*; white symbols: urban wastewater; black symbols: hospital urine; j: 10 A m^{-2}. (b) Kinetic constants calculated for the removal of ARB by electrolysis with diamond anodes. j: 10

A m^{-2}; (■) *E. coli*; (▲) *K. pneumoniae*; (●) *E. faecalis*; (□) urea; (Δ) creatinine; (○) uric acid.

As can be observed, the disinfection efficiency depends on the nature of the ARB and on the complexity of the aqueous matrix. The population of all microorganisms decreases with the applied electric charge and the complete disinfection is reached at values below 0.15 Ah dm^{-3}. This is due to the action of disinfectant species generated during electrolysis. The occurrence of these species is detailed later. Comparing results of the three ARB tested, *E. faecalis* shows a higher depletion efficiency followed by *K. pneumoniae* and, finally, *E. coli*. This difference can be related to the cell morphology of each bacteria since *E. faecalis* is a gram-positive bacterium whereas *K. pneumoniae* and *E. coli* are gram-negative bacteria. Specifically, gram-positive bacteria present a cytoplasmic membrane and a cell wall in its structure, while gram-negative bacteria are constituted by a cytoplasmic membrane, the cell wall and an additional external membrane [21]. This supplementary membrane in gram-negative bacteria could be the responsible for the higher resistance against the disinfection process.

The differences observed also depend on the water matrix. Thus, the disinfection efficiency is higher when working with urban treated wastewater since the complete removal of microorganisms is attained at electric charges below 0.05 Ah dm^{-3}, regardless the bacteria tested. This marked influence of water matrix on the electrochemical disinfection can be related to a competitive oxidation of other compounds during the treatment. In this context, hospital urine also contains organic compounds in high concentration that consume electrons and oxidants for being oxidized: urea, creatinine, and uric acid [18]. On the contrary, urban treated wastewater may contain humic acids as organic load but in low concentration in comparison with the other organics from urine. Therefore, the oxidation of this species is negligible during the electrochemical disinfection, but this is relevant in the case of hospital urine [11]. Hence, the lower disinfection efficiency during the treatment of hospital urine may be related to the simultaneous oxidation of microorganisms and organics.

Experimental data were fitted to a first order kinetics model and the resulting constants are shown in Figure 1b. For comparison purposes, kinetic constants from the degradation of organics contained in hospital urine have been also plotted. As can be observed, the disinfection rate of urban treated wastewater is higher than that of hospital urine, being the kinetic constants within the range 0.81-1.02 and 0.13-0.18 min^{-1} for both media, respectively. The ratio $k_{\text{wastewater}}/k_{\text{urine}}$ (which compares disinfection in wastewater and urine media) confirms that the disinfection rate of *E. faecalis*, *K. pneumoniae* and *E. coli* in urban treated wastewater is 7.02, 6.27 and 5.43 times faster than in urine, respectively. Additionally, the resulting kinetic values for urea, creatinine and uric acid are some order of magnitude lower ($k < 0.003\ \text{min}^{-1}$), but, despite this, it confirms the existence of competitive oxidation between bacteria and organics contained in hospital urine and reveals the importance of the water matrix on the disinfection process.

In this point, it is important to remark that the electrochemical disinfection mainly takes place by the action of disinfectant species electrochemically generated during the process [7]. Therefore, the type and concentration of inorganic species present in aqueous matrix that are susceptible to form strong disinfectants will mark the efficiency of the process. From the disinfection viewpoint, chloride is the ion with the most interest because its oxidation leads to hypochlorite, which shows a high disinfection capacity. Additionally, it can react with ammonium, typically contained in both effluents, to form chloramines which are also disinfectants. On the other hand, hypochlorite can also react with organics present in the reaction media (chlorination reaction) or it can be further oxidized to other undesirable chlorine compounds in high oxidation state (chlorate and perchlorate) [22], whose presence in treated water is not desirable. For this reason, the evolution of chlorine species was monitored during the process and results are plotted in Figure 2.

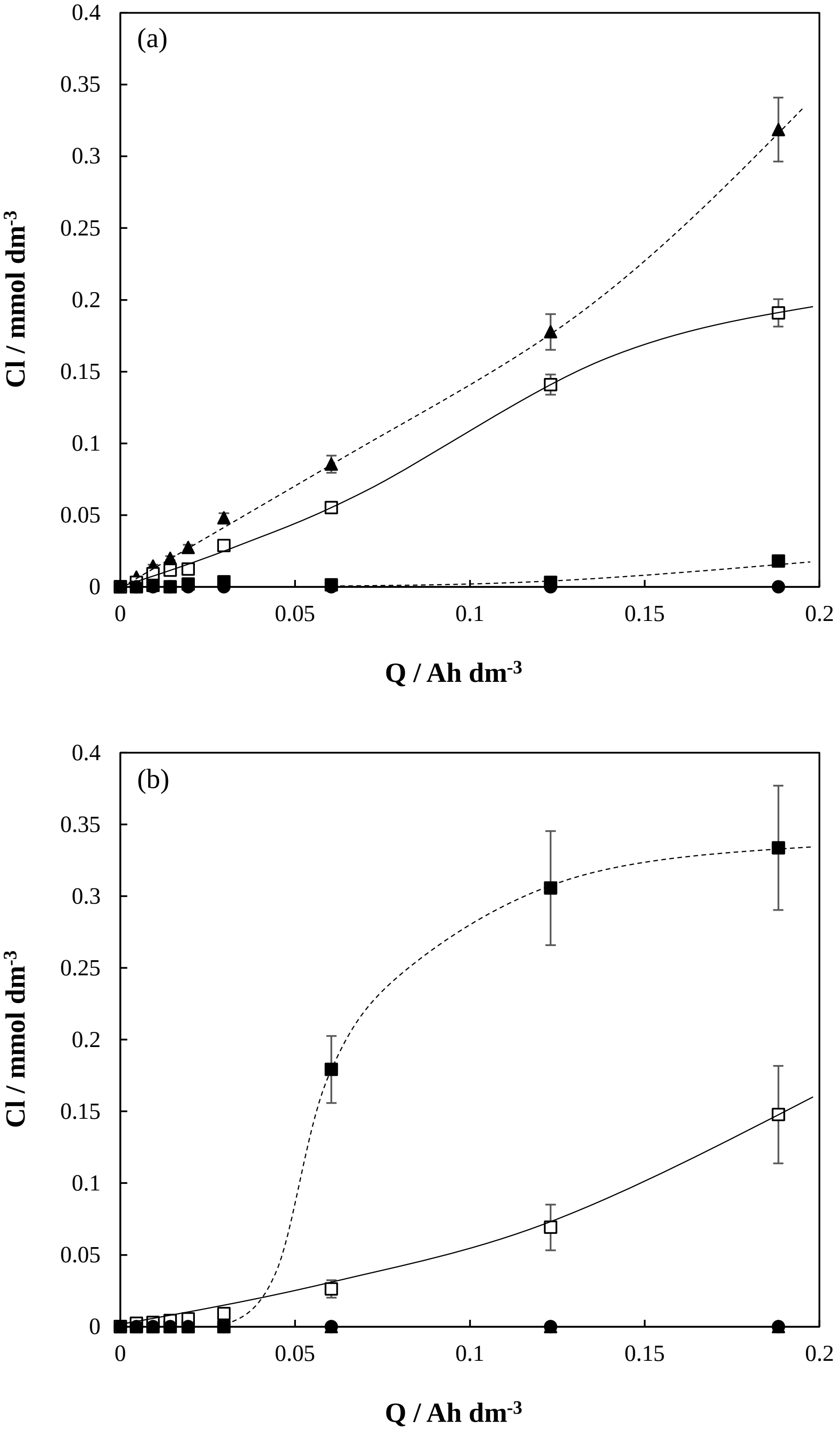

Figure 2. Influence of water matrix on chlorine speciation during the removal of antibiotic resistant bacteria by electrolysis with diamond anodes in (a) urban treated

wastewater and (b) hospital urine. (■) Hypochlorite; (▲) chlorate; (●) perchlorate; (□) chloramines. j: 10 A m^{-2}.

As can be observed, the concentration of chlorine species increases with the applied electric charge, although the trend and the maximum concentration depend on the water matrix composition. In the case of urban treated wastewater (Figure 2a), the concentration of hypochlorite is not relevant (lower than 0.02 mmol dm^{-3}) in comparison with chloramine and chlorate ones. This indicates that once hypochlorite is generated by the oxidation of chlorides, it rapidly reacts. According to disinfection results shown in Figure 1 and to chemical disinfection tests carried out (data not shown), hypochlorite reacts rapidly with bacteria (kinetic constants ranging from 0.81 to 1.02 min^{-1}). Then, once bacteria have decreased the hypochlorite electrogenerated continuous to react with ammonium contained in the treated wastewater, and chloramines are accumulated in the reaction media. The trend observed reveals that the generation rate of these species decreases at higher electric charges which may be related to a limited concentration of ammonium in wastewater or to the promotion of hypochlorite to chlorate (competitive reaction) whose generation rate increases at the end of the treatment. Chlorate is a hazardous species that should be avoided.

On the other hand, in urine media the trend of chlorine species is different, and this could be related to its higher complexity (see Table 1). As observed in Figure 2b, in the first stages of the process (electric charges below 0.05 Ah dm^{-3}), the accumulation of chloro-species is not favored, and only chloramines start to be detected in the solution (but in very low concentration). The formation of chloramines only can come from the breakpoint reaction which requires the presence of hypochlorite. Then, it confirms that hypochlorite is formed, but its rate of disappearance is greater than its rate of generation. Chloramines profile suggests that its concentration could be increased by the continuous nitrogen release from the oxidation of organics. Unlike urban treated wastewater, the concentration of organics naturally contained in urine is not negligible and they can consume part of the hypochlorite generated. This, together with the hypochlorite used in the disinfection, may justify the delay in hypochlorite accumulation observed in urine. Additionally, the higher initial concentration of chlorides in urine (475.52 *vs.* 148.22 mg dm^{-3}) may explain the higher concentration of hypochlorite accumulated at the end of the process. Furthermore, it is important to highlight the null concentration of chlorate registered in hospital urine. This may indicate that while there are other species in the reaction media that may be attacked (such as uric acid, urea or creatinine), the hypochlorite is not available to be oxidized to chlorate. These results are quite relevant and point out that the formation of hazardous chlorine by-products can be avoided if the removal of ARB by electrodisinfection with diamond anodes is directly carried out in hospital urine since the formation of hypochlorite and chloramines is mainly promoted as chlorine species. In addition, from a technical and economic point of view, another advantage of disinfecting hospital urine is its low generation volume compared to that of treated urban wastewater, since hospital urine represents around 2-3% of the total volume of hospital effluents [23] and these, in turn, represent around 20% of the volume of urban wastewater [24]. Therefore, although the electric charge necessary for the complete elimination of ARBs is around 6 times higher in urine, the volume to be treated is 5 log units lower, so that both the investment and operation costs would be much lower. This makes direct electrodisinfection of hospital urine an excellent option from a techno-economic point of view to decrease the presence of ARB in the aquatic environment.

4. Conclusions

The following conclusions can be drawn from this work:

- ARB removal efficiency is lower in hospital urine because of the existence of other competitive oxidation reactions during electrolysis.
- Gram-positive bacteria are easier to deplete than gram-negative bacteria.
- The occurrence of hazardous disinfection by-product is avoided when the removal of ARB is carried out in hospital urine. The production of hypochlorite and the subsequent formation of inorganic chloramines are favored over production of chlorates in this complex matrix.

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