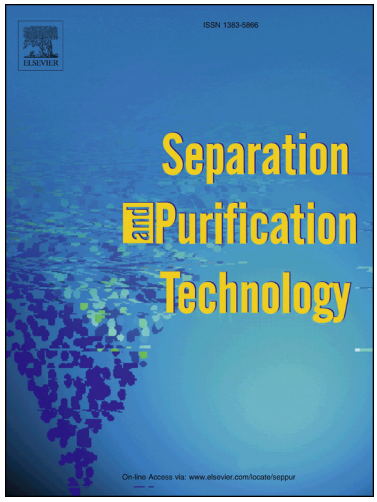

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Effect of electrogenerated hydroxyl radicals, active chlorine and organic matter on the electrochemical inactivation of *Pseudomonas aeruginosa* using BDD and dimensionally stable anodes

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Abstract

In this work, the disinfection of 100 mL of 10^6 CFU mL^{-1} *Pseudonomas aeurigonosa* suspensions at pH 5.8 by electrochemical oxidation at 33.3 mA cm^{-2} is reported. The undivided electrolytic cell was equipped with either a boron-doped diamond (BDD) or an IrO_2-based or RuO_2-based dimensionally stable anode and a stainless steel cathode. Physisorbed hydroxyl radicals M($^{\bullet}$OH) formed from anodic water oxidation and active chlorine generated from anodic Cl^- oxidation were the main oxidizing species in pure Na_2SO_4 medium and in the presence of NaCl, respectively. A faster inactivation was always found using the dimensionally stable anodes. In 7 mM Na_2SO_4, this behavior was associated to the much larger adsorption of the bacteria onto the anode, which accelerated the M($^{\bullet}$OH)-mediated oxidation and inactivation of the cells. The inactivation rate was strongly enhanced in 7 mM Na_2SO_4 + 1 mM NaCl due to the larger oxidation power of active chlorine compared to that of M($^{\bullet}$OH). The effect of NaCl concentration and current density on the disinfection process was examined with BDD and the best performance was obtained in 7 mM Na_2SO_4 + 7 mM NaCl at 8.3 mA cm^{-2}, with total inactivation in 2 min and energy consumption of 0.059 kWh m^{-3}. The addition of paracetamol in 7 mM Na_2SO_4 medium inhibited the disinfection at short electrolysis time regardless of the anode, owing to the preferential action of M($^{\bullet}$OH) on this pollutant. For BDD, the inactivation rate rose over time at higher drug content due to the generation of greater amounts of toxic by-products. For the IrO_2-based anode, the progressive formation of toxic and less adsorbable by-products enhanced the process over time, giving rise again to a quicker total disinfection compared to that with BDD.

Keywords: Active chlorine; Electrochemical disinfection; Hydroxyl radical; Paracetamol; *Pseudomonas aeruginosa*

1. Introduction

Over the last fifteen years, the effective removal of many toxic and recalcitrant organic pollutants from wastewater by electrochemical advanced oxidation processes (EAOPs) has been exhaustively examined [1-6]. The EAOPs are environmentally friendly methods because the main reactant is the electron, which is a clean reagent, and no additional species or just small amounts of inocous ones are futher needed. These methods present important technological advantages such as ease of automation, versatility, high efficiency and safety since they operate under mild conditions. The common feature of all EAOPs is their ability to produce strong oxidants like reactive oxygen species (ROS) on site. The most significant ROS is the hydroxyl radical ($^{\bullet}$OH), which has a high standard reduction potential (E^{o} = 2.80 V/SCE) that facilitates its reaction with many organics up to their mineralization [3-6].

The most developed EAOP is electrochemical oxidation (EO), also called electro-oxidation or anodic oxidation [1,5]. It involves the degradation of organic pollutants from a contaminated solution contained in the electrolytic cell either by direct oxidation at the anode surface or by mediated reaction with strong oxidizing agents generated on site. In chloride-free solutions, physisorbed hydroxyl radicals (M($^{\bullet}$OH)) are produced as intermediate of O_2 evolution from water discharge at the anode M as follows [1,7]:

$$M + H_2O \rightarrow M(^{\bullet}OH) + H^{+} + e^{-} \quad (1)$$

The performance of EO depends on the kind of anodic material tested. For the so-called active anodes, such as Pt and dimensionally stable anodes like IrO_2 and RuO_2, the mineralization of organics is usually low because most of the physisorbed M($^{\bullet}$OH) are converted into the chemisorbed "superoxide" MO with poor oxidization power [8,9]. This transformation is minimized in the so-called non-active anodes like PbO_2, SnO_2 and boron-doped diamond (BDD), which present a large ability to degrade the organic load [1,5,6]. It

has been found that BDD thin-film electrodes are the most powerful anodes in EO because they exhibit the highest O_2-overvoltage and a very weak interaction with $^{\bullet}OH$ and organics, thus favoring the complete mineralization of aromatics and linear aliphatic compounds [10-18]. Nevertheless, the main drawbacks of the BDD anode are its high cost and the simultaneous production of other weaker oxidants like ozone and peroxodifulfate ($S_2O_8^{2-}$) ion in sulfate medium [7,19].

A much more complex EO process takes place in the presence of chloride ions, since the organic molecules can be attacked by active chlorine (Cl_2/HClO/ClO^-) produced via reactions (2)-(4), in competence with M($^{\bullet}OH$) [2,3,20-22].

$$2\ Cl^- \rightarrow Cl_{2(aq)} + 2\ e^- \quad (2)$$

$$Cl_{2(aq)} + H_2O \leftrightarrows HClO + Cl^- + H^+ \quad (3)$$

$$HClO \leftrightarrows ClO^- + H^+ \quad (4)$$

The predominant species is $Cl_{2(aq)}$ (E^o = 1.36 V/SHE) up to pH 3.0, HClO (E^o = 1.49 V/SHE) at pH 3-8 and ClO^- (E^o = 0.89 V/SHE) at pH > 8.0. Consequently, HClO is the most powerful active chlorine species and thus, the most successful mediated oxidation of organics in such medium is likely to occur under slightly acidic and neutral conditions. Active chlorine is expected to be more extensively produced at dimensionally stable anodes, rather than at BDD, because of its larger electrocatalytic ability regarding reaction (2) [2,3].

Closely related to the destruction of organic matter by EO, the application of this EAOP to the inactivation of pathogenic microorganisms present in water has been investigated as well. This mainly occurs by the oxidation of the compounds of the cell wall, which damages them and subsequently causes the inactivation of other essential molecules of the bacteria [23-25]. The electrochemical disinfection has been successful for the treatment of urban wastewater, pools and spas, among others [26-28]. The inactivation ability of EO depends on

the nature of the anode and the ions contained in solution. The antimicrobial action of physisorbed BDD($^{\bullet}$OH) originated from reaction (1) in chloride-free medium has been well proven [25,29-33]. A quicker disinfection is usually achieved in the presence of chloride ions because of the rapid attack of active chlorine species, being Pt and dimensionally stable anodes more efficient than BDD [26,27,34-37]. However, most of the studies have been focused on the inactivation of *Escherichia coli* as process indicator [31,33,36], with much less attention on other hazardous bacteria such as *Pseudomonas aeruginosa*. Pseudomonas are aerobic, rod-shaped Gram-negative bacteria, which are distinguished by their versatile metabolism. *Pseudomonas aeruginosa* is ubiquitous in water and soil, also detected in plants and animals. It is an opportunistic pathogen, related to external otitis, keratitis, dermatitis and even pneumonia. It is also the cause of infections in burned people and cystic fibrosis patients, being the fifth most frequent cause of nosocomial infections, especially due to surgical interventions and septicemia among immunocompromised or traumed people [38,39]. Nonetheless, most of the infections by Pseudomonas result from contact with contaminated water, either in natural environments or in aquatic facilities such as swimming pools or hot tubs, where they often form biofilms that act as a reservoir [40]. Currently, major concerns arise from the gradually greater resistance of these bacteria to antibiotics. On the other hand, it is well known that pharmaceuticals in water can exert toxic effects on aquatic organisms and consumers [2], but there is scarce information on their interaction with bacteria resulting from competitive consumption of oxidants during inactivation treatments [36]. A common antiinflammatory and analgesic pharmaceutical widely consumed in human and veterinary medicine is paracetamol (PCM, *N*-(4-hydroxyphenyl)acetamide, $C_8H_9NO_2$). This drug has been detected in sewage wastewater treatment plants up to 6 μgL^{-1} and its effective mineralization by EO in sulfate and chloride solutions with a BDD anode has been reported elsewhere [41,42].

This paper aims to clarify the electrochemical disinfection of *Pseudomonas aeruginosa* suspensions in sulfate and mixed sulfate + chloride media at natural pH near 5.8. The bactericidal ability of electrogenerated M($^{\bullet}$OH) and active chlorine in EO was examined using BDD, IrO_2-based and RuO_2-based anodes under analogous conditions. The influence of several PCM contents on bacteria inactivation in sulfate medium was also assessed.

2. Experimental

2.1. Chemicals

Paracetamol (> 99% purity) was supplied by Merck. Sodium chloride and sodium sulfate used for the electrolytic experiments were of analytical grade purchased from Panreac Química. All the solutions were prepared with high-purity water from a Millipore Milli-Q system with resistivity > 18 MΩ cm.

2.2. Bacteria and culture

Strains of the rod-shaped Gram-negative bacterium *Pseudomonas aeruginosa* ATTC 15442 have been employed in this work. Bacteria were cultured in Trypticasein Soy Agar (TSA) plates, purchased from Laboratorio Conda, at 37 ºC for 24 h. The cells were subsequently spiked in 2 mL of 7 mM Na_2SO_4, centrifuged at 14,000 rpm for 2 min and washed twice with 1 mL of 7 mM Na_2SO_4. The resulting pellet was resuspended in 1 mL of the same electrolyte so as to give an optical density at 600 nm (O.D. 600) of 0.7±0.1, related to about 10^8 colony-forming units per mL (CFU mL^{-1}).

2.3. Electrolytic trials

An undivided, two-electrode cylindrical tank reactor of 150 mL capacity was used for all electrolytic trials. The cell was surrounded with a jacket for circulation of thermostated water to keep the solution temperature at 25 ºC. The anode was either a BDD thin-film electrode

purchased from NeoCoat (La-Chaux-de-Fonds, Switzerland), a Ti/RuO_2-based plate or a Ti/IrO_2-based plate, both supplied by NMT Electrodes (Pinetown, South Africa). In all the assays, the cathode was a stainless steel (AISI 304) sheet. The area of all electrodes was 3 cm^2 and the interelectrode gap was close to 1 cm. All experiments were performed under galvanostatic conditions, using an Amel 2053 potentiostat-galvanostat to set the current density (*j*) and a Demestres 601BR digital multimeter to measure the cell voltage. Before the assays, the surface of all anodes was cleaned by polarization in 50 mM Na_2SO_4 at j = 100 mA cm^{-2} for 180 min.

The disinfection assays were carried out with 100 mL of aqueous solutions containing 7 mM Na_2SO_4 alone or in the presence of 1, 3 or 7 mM NaCl at ca. pH 5.8, always under vigorous stirring at 800 rpm with a magnetic PTFE bar. In the former medium, PCM contents up to 157 mg L^{-1} (100 mg L^{-1} TOC) were added in som cases to check the influence of organic matter. Each solution was spiked with a concentrated bacterial suspension of 10^8 CFU mL^{-1} to obtain a suspension with 10^6 CFU mL^{-1}. Before each trial, the cell was cleaned with a H_2O_2:H_2SO_4 mixture for 10 min, rinsed with ultrapure water and dried in an oven at 80 ºC. The electrodes were immersed in ultrapure water at 100 °C for 10 min, followed by air drying.

2.4. Analytical methods

The O.D. 600, pH and electrical conductance of bacterial suspensions were determined with a Camspec M108 spectrophotometer, a Crison GLP 22 pH-meter and a Metrohm 644 conductometer, respectively. Total organic carbon (TOC) of initial and final electrolyzed suspensions was obtained by injecting 50 μL aliquots into a Shimadzu TOC-VCSN. Reproducible TOC values with an accuracy of ±1% were always found. Active chlorine was determined by the *N*,*N*-diethyl-*p*-phenylenediamine (DPD) colorimetric method using a Shimadzu 1800 UV/Vis spectrophotometer selected at λ = 515 nm [43].

The inactivation of *Pseudomonas aeruginosa* strains was followed by withdrawing aliquots (1 mL) at different time periods for 30-60 min of electrolysis. Samples obtained from sulfate medium were diluted in 7 mM Na_2SO_4, whereas those of mixed media were diluted in 7 mM Na_2SO_4 + 3% $Na_2S_2O_3$ solution immediately after collection to neutralize the bactericidal effect of residual active chlorine. In both cases, the samples were cultured in duplicate on TSA plates and incubated at 37 ºC for 24 h. The reduction of culturability was measured as log (N_t/N_0), being N_t the CFU value at time t and N_0 the initial CFU content. The theoretical detection limit was 1 bacterium per mL. All the trials were made in triplicate and average values of log unit reduction are presented in figures, along with the error bars related to a 95% confidence interval.

3. Results and discussion

3.1. Electrochemical inactivation in sulfate medium

A first study on the disinfection of 100 mL of a 10^6 CFU mL^{-1} *Pseudomonas aeruginosa* suspension by EO was performed in 7 mM Na_2SO_4 using a BDD, RuO_2-based or IrO_2-based anode and a SS cathode at j = 33.3 mA cm^{-2} for 60 min. In these comparative assays, the cell voltage was about 16.9 V for the former anode and about 15.6 V for the latter ones, with only a change of ± 0.5 V as maximal during the trials. The low conductivity of the starting solution was about 1.8 mS cm^{-1} and remained practically invariant, whereas the initial pH ~ 5.8 and initial TOC close to 3.1 mg L^{-1} underwent slight decays of 0.5 units and 0.8 mg L^{-1} as maximal, respectively. All these quite stable parameters suggest that the mineralization of bacterial cells during the EO treatment was not significant. Moreover, a preliminary test informed about the poor inactivation attained for bacterial suspensions in 7 mM Na_2SO_4 in the absence of current supply.

Fig. 1 depicts a gradual reduction of log (N_t/N_0) with electrolysis time in all cases, attaining total inactivation (i.e., 7 log-unit decay) after 60 min. At 15 min, however, a very poor drop of less than 1 log-unit was achieved for the BDD anode, whereas the log (N_t/N_0) was reduced by 4.5 units using the IrO_2-based one and by a slightly higher value of 5.3 units using the RuO_2-based one. The inactivation rate was so high for the two dimensionally stable anodes that they led to a reduction of 7 log-units at 30 min, but the use of the BDD anode only allowed the removal of 4.6 log units at that time. Note that the accuracy of the log (N_t/N_0) decay is limited by the sensitivity of the analytical method used to determine *Pseudomonas aeruginosa*, thus yielding a plateau at too low bacteria content. The results of Fig. 1 reveal that EO at j = 33.3 mA cm^{-2} yielded > 99.999% of inactivation (> 5 log-unit destruction) after 15, 30 and 45 min of electrolysis with a RuO_2-based, IrO_2–based and BDD anode, respectively. Under these conditions, the disinfection process involves pre-eminently the action of physisorbed M($^{\bullet}$OH) formed from reaction (1), along with other ROS such as H_2O_2, O_3 and $O_2^{\bullet-}$ [25,29,30] for all anodes, and other weak oxidants like $S_2O_8^{2-}$ ion for BDD [1,3]. These oxidizing species damage the cell membranes, altering their permeability and finally affecting other essential molecules of the bacteria [23,33]. Our results could seem rather unexpected because it is well known that the BDD electrode is a non-active anode with low adsorption ability that produces much greater amounts of M($^{\bullet}$OH) compared to the two active dimensionally stable materials [1,3,4]. The superior bactericidal power of the latter anodes (see Fig. 1) can then be ascribed to a much larger adsorption of the cells onto their surface compared to that occurring on BDD, thus favoring the attack of the adsorbed oxidizing species over their structure and eventually enhancing their inactivation very remarkably.

3.2. Electrochemical disinfection in mixed sulfate + chloride media

Once examined the inactivation of *Pseudomonas aeruginosa* in suspensions with a pure sulfate salt, the effect of electrogenerated active chlorine was investigated employing a mixed medium containing 7 mM Na_2SO_4 + 1 mM NaCl. This solution presented a conductivity of about 1.9 mS cm^{-1} and pH ca. 5.8, which are similar values to those obtained after 30 min of electrolysis at j = 33.3 mA cm^{-2} regardless of the anode used.

First, the ability of the different electrolytic systems to generate active chlorine (mainly in the form of HClO under the current experimental conditions) in the bulk in the absence of the bacterial suspension was assessed for 15 min of electrolysis. Fig. 2 shows a progressive rise in active chlorine concentration for all the anodes tested, attaining final values close to 0.3, 1.4 and 5.2 mg L^{-1} for EO with a BDD, IrO_2-based and RuO_2-based anode, respectively. The superiority of the latter anode can be related to its larger electrocatalytic ability to oxidize Cl^- ion from reaction (2) under the tested conditions. This agrees with previous results by Jeong et al. [34], who reported that the accumulation rate of active chlorine using dimensionally stable anodes such as RuO_2, IrO_2 and Pt-IrO_2 was much higher than that with BDD. The low contents of this species achieved up to 5 min (see Fig. 2) can be explained by the existence of parasitic reactions, as for example the formation of chlorate and perchlorate from HClO oxidation, which are particularly important in the case of the BDD anode [2-4,24]. The determination of active chlorine by the DPD method was not made in the presence of bacteria since a large variability was expected depending on its consumption upon attack over the cells in the solution bulk [24,27,36].

Fig. 3 highlights the comparative log (N_t/N_0) decay with electrolysis time for the EO treatment of 100 mL of 10^6 CFU mL^{-1} bacterial suspensions in 7 mM Na_2SO_4 + 1 mM NaCl using the three types of anode at j = 33.3 mA cm^{-2}. Total inactivation with a 7-log reduction was rapidly obtained after only 5 min of treatment for both, IrO_2-based and RuO_2-based anodes, meaning that the active chlorine content generated under such conditions was high

enough to oxidize and alter the wall of all the cells within a very short time. In contrast, the use of BDD yielded a much slower inactivation rate and more than 15 min were needed to inhibit the culturability of all the bacteria in suspension. Comparison of Fig. 1 and 3 allows inferring a much quicker decay of log (N_t/N_0) in the mixed solution than in the pure sulfate medium. For example, the CFU content in 7 mM Na_2SO_4 decreased by 0.8, 4.5 and 5.3 log units in 15 min using BDD, IrO_2-based and RuO_2-based anodes, whereas it experienced a much larger drop of 4.7 log units for the former anode and near 7 log units for the two latter ones in 7 mM Na_2SO_4 + 1 mM NaCl. This means that electrogenerated active chlorine possesses much larger oxidation power and, consequently, much larger disinfection ability than ROS generated as unique oxidants in pure sulfate medium. In the latter case, the bacterial adsorption onto the anode surface played a crucial role on inactivation trends because the M($^{\bullet}$OH) only acts in the anode vicinity, whereas active chlorine mainly acts in the whole bulk. Worth noting, Cl^- ion is detrimental for M($^{\bullet}$OH) formation due to the following parasitic destruction reaction [16]:

$$Cl^- + M(^{\bullet}OH) + H^+ \rightarrow Cl^{\bullet} + M + H_2O \quad (5)$$

Therefore, in chlorinated media, apart from the generation of active chlorine as main oxidant, other chloro radicals like $Cl^{\bullet}$, $ClOH^{\bullet-}$ and $Cl_2^{\bullet-}$ can contribute to bacterial inactivation [16].

The aforementioned findings bring to consider that Cl^- concentration and j can be key parameters to disinfect the *Pseudomonas aeruginosa* suspensions by EO. To corroborate this, mixed solutions of 7 mM Na_2SO_4 with 1, 3 or 7 mM NaCl were comparatively electrolyzed at j = 33.3 mA cm^{-2}, and the 7 mM Na_2SO_4 + 7 mM NaCl solution was also treated at j = 8.3 mA cm^{-2}. These assays were carried out with the BDD/SS system, despite its lower oxidation power, aiming to better observe the influence of such parameters. The cell voltage decreased

from ca. 15.1 to 13.2 V for the mixed solutions at increasing NaCl concentration from 1 to 7 mM due to the gradually higher conductivity (from 1.9 to 2.4 mS cm^{-1}). No significant variation of cell voltage, conductivity, pH (near 5.8) and TOC (close to 3.1 mg L^{-1}) was found during the 30-min treatments at 33.3 mA cm^{-2}. The same behavior was observed for the experiment at j = 8.3 mA cm^{-2}, with a cell voltage of 7.1 V.

Fig. 4a and b exemplify the accumulation of active chlorine up to 15 min of electrolysis for the above conditions but in the absence of bacteria. A growing final content of 0.29, 0.37 and 15.8 mg L^{-1} of this species was accumulated in the bulk using the mixed solution with 1, 3 and 7 mM NaCl, respectively, always operating at j = 33.3 mA cm^{-2}. The large upgrade of active chlorine production when using 7 mM instead of 3 mM NaCl can be ascribed to a high increase in rate of reaction (2) favoring the generation of HClO from reaction (3), plausibly due to the concomitant decay in cell voltage of the system. This behavior was confirmed by the high accumulation (i.e., 10.4 mg L^{-1} active chlorine) also found for the mixed solution with 7 mM NaCl when a lower j of 8.3 mA cm^{-2} was used, as can be seen in Fig. 4a. All these findings suggest that this medium with much higher active chlorine accumulation should yield faster inactivation of the 10^6 CFU mL^{-1} *Pseudomonas aeruginosa* suspension upon EO treatment. This assumption was corroborated when the corresponding log (N_t/N_0) decays were measured.

Fig. 5 depicts a complete inactivation of the strain in only 2 min using the 7 mM Na_2SO_4 + 7 mM NaCl medium, regardless of the j value applied, as a result of the excessive active chlorine content generated. In contrast, the process was slower for 3 mM NaCl and 7 log units were reduced at 10 min of electrolysis due to lower production of active chlorine. The use of the smallest NaCl concentration (1 mM NaCl) led to a slower destruction of the cell walls and a time as long as ~ 30 min was required for total inactivation. These results indicate that the NaCl concentration is the main parameter that determines the performance of the EO

disinfection process, rather than *j* since a very similar tendency was obtained at 33.3 and 8.3 mA cm^{-2} when 7 mM NaCl was utilized with a BDD anode. This decrease in *j* entailed a strong drop of the required energy consumption for complete inactivation, from 0.440 to 0.059 kWh m^{-3}, which is a very interesting feature for practical application of this technology. Even lower energy consumption could be achieved with a powerful, dimensionally stable anode. This can be deduced from the fact that, in 7 mM Na_2SO_4 + 1 mM NaCl, this parameter was 0.983 kWh m^{-3} when using the best anode (i.e., the RuO_2-based one) as a result of a cell voltage of 11.8 V at j = 33.3 mA cm^{-2}. This value was much lower than 7.55 kWh m^{-3} obtained with the BDD one under the same conditions (see Fig. 3).

Interestingly, the disinfection of *Pseudomonas aeruginosa* suspensions by EO has been previously reported only with BDD/BDD flow systems, which found great difficulty to promote the inactivation. The authors assumed that BDD was the best electrode to generate oxidizing species to attack the microorganism, which does not agree with the results of our work since dimensionally stable anodes show a best performance in both, chloride-free and chlorinated solutions. Thus, Griessler et al. [32] described reduction of near 4 log units of culturability starting from a 10^6 CFU mL^{-1} bacterial suspension in tap water containing 7.8 mg L^{-1} SO_4^{2-} + 7.0 mg L^{-1} Cl^- after 30 min of electrolysis. A high *j* of 140 mA cm^{-2} was applied, resulting in an enormous cell voltage of 230 V owing to the extremely low conductivity of the medium. More recently, Rajab et al. [37] described the disinfection of 10 L of 10^7-10^8 CFU mL^{-1} bacterial suspensions under recirculation at 1.2 L h^{-1}. In pure water, a log (N_t/N_0) removal of 3.2 log units was obtained operating at 42 mA cm^{-2} for 60 min, whereas total inactivation was obtained in 15 min at 167 mA cm^{-2}. Better results were found upon addition of 20 mg L^{-1} Cl^- (5.6 mM) to the solution, reaching culturability reductions of ~ 6 log units after 60 and 5 min at 42 and 167 mA cm^{-2}, respectively. Our results have also

shown such an improved inactivation with the BDD anode in the presence of Cl^- ion (see Fig. 1 and 3).

3.3. Effect of organic matter on the electrochemical disinfection process

In most real cases, the microorganisms are present in water along with organic matter, including emerging pollutants like pharmaceuticals. Hence, the influence of such a potential oxidant scavenger on the competitive consumption of ROS was assessed by selecting PCM as model organic compound. Preliminary control experiments of suspensions with 10^6 CFU mL^{-1} *Pseudomonas aeruginosa* and 157 mg L^{-1} PCM (corresponding to 100 mg L^{-1} TOC) in 7 mM Na_2SO_4 at ca. pH 5.8 did not show any decrease in culturability over 60 min, thereby evidencing the inoquous character of PCM for the tested bacterial strain. EO experiments were then performed with different PCM concentrations using a BDD or an IrO_2-based dimensionally stable anode at j = 33.3 mA cm^{-2} for 60 min. The assays were not made in chloride medium since the expected much faster bacterial inactivation would prevent a good evaluation of the effect of organics.

Fig. 6a highlights the slower disinfection of the bacterial suspensions in the presence of PCM as compared to the EO in its absence, using a BDD anode, as expected from the non-selective attack of the electrogenerated oxidants (ROS an $S_2O_8^{2-}$ ion) over both, the microorganisms and the organic matter. However, it is noticeable that the log (N_t/N_0) removal was enhanced when the PCM content rose from 27 to 157 mg L^{-1} or when it decreased from 27 to 0 mg L^{-1}. In other words, the inactivation rate profile showed a minimum at 27 mg L^{-1} PCM. At 45 min, for example, an inactivation of 5.8, 4.0, 1.7, 2.8, 4.6 and 5.2 log units was obtained at 0, 11, 27, 39, 79 and 157 mg L^{-1} PCM, respectively. This trend suggests that the action of the oxidizing species was gradually more focused on PCM and its by-products when increasing the content up to 27 mg L^{-1}, thus yielding a slower inactivation. Since a similar amount of oxidants is expected to be produced at the same j of 33.3 mA cm^{-2}, the subsequent

progressive disinfection enhancement from 27 to 157 mg L^{-1} PCM at a given time can be related to the formation of more toxic organic by-products, which inactivate the cells to a gradually larger extent. In this way, at 60 min of electrolysis, total disinfection was achieved in the absence of organics as well as with the highest PCM concentration. All the other solutions attaining > 6 log-unit reduction, except that with 27 mg L^{-1} that only reached a 5-log decay due to the smaller generation of toxic by-products. The formation of toxic hydroquinone and *p*-benzoquinone during the EO treatment of PCM has been reported by some of us elsewhere [41], and such by-products could be responsible for the acceleration of the bacterial disinfection. Our results also agree with the progressively greater abatement of solution TOC, being 1.2, 2.6, 3.5, 8.8 and 19.6 mg L^{-1} at rising PCM contents of 11, 27, 39, 79 and 157 mg L^{-1}, respectively. This tendency can be accounted for by the larger efficiency of oxidants to destroy the organic pollutants, thanks to the concomitant deceleration of their parasitic reactions [2-4,41].

The comparative results using an IrO_2-based anode are presented in Fig. 6b, and they differ significantly from those obtained with BDD (see Fig. 6a). Total disinfection for the former anode was already achieved at 30 min in the absence of organic matter and at 60 min for all the other suspensions containing up to 157 mg L^{-1} PCM, making the inactivation process more efficient than that with BDD. Nevertheless, a smaller formation of toxic by-products is expected due to its lower oxidation ability to mineralize the organic by-products since TOC was reduced by less than 2 mg L^{-1} in all cases. As stated above, the faster inactivation attained with the IrO_2-based anode compared to BDD can be associated with the better adsorption of the cells onto its surface that favors the oxidation of their walls with M($^{\bullet}$OH). The lower inactivation rate in the presence of PCM could then be related to the simultaneous reaction between M($^{\bullet}$OH) and either the adsorbed cells, PCM or its by-products. Results of Fig. 6b suggest the pre-eminent oxidation of adsorbed PCM at a short electrolysis

time of 15 min, which hampered the disinfection process in all cases. In contrast, the gradual production of larger amounts of less adsorbable by-products over time with increasing PCM content favored the bacterial adsorption, thereby enhancing the inactivation rate when going from 11 to 157 mg L^{-1}, as can be observed at 30 and 45 min of electrolysis. Furthermore, the concomitant larger toxicity of reaction by-products was beneficial, favoring the disinfection the bacterial suspensions. Such toxic organics can be gradually degraded under the action of M($^{\bullet}$OH), ending in a total disinfection along with a quantitative decontamination of the water.

4. Conclusions

It has been shown that a *Pseudomonas aeruginosa* strain spiked at 10^6 CFU mL^{-1} in a chloride-free aqueous medium at pH 5.8 can be inactivated by EO at different rates depending on the anodic material. Total inactivaction was achieved in 60 min using a BDD anode due to the oxidation and damage of the cell wall pre-eminently by electrogenerated physisorbed M($^{\bullet}$OH). The process became much faster using IrO_2- or RuO_2-based anodes since the greater adsorption of microorganisms onto their surface enhanced their reaction with M($^{\bullet}$OH), attaining complete inactivation in 30 min. In the presence of NaCl, HClO caused a much rapid disinfection, requiring 5 and < 30 min for complete inactivation with both dimensionally stable anodes and BDD, respectively, at 33.3 mA cm^{-2}. With the BDD/SS system, the concentration of active chlorine grew at higher NaCl content from 1 to 7 mM and *j* from 8.3 to 33.3 mA cm^{-2}. The best performance was found in 7 mM Na_2SO_4 + 7 mM NaCl at 8.3 mA cm^{-2}, with complete disinfection in only 2 min and energy consumption as low as 0.059 kWh m^{-3}. The inactivation was always more favorable using a dimensionally stable anode. The addition of PCM in Na_2SO_4 medium revealed the preferential destruction of this compound from 27 mg L^{-1} using a BDD anode, although the inactivation also rised due to the gradually larger amounts of toxic by-products. With the IrO_2-based anode, the cell adsorption was

inhibited at the beginning of the electrolysis, but the inactivation was further accelerated over time by the formation of less adsorbable toxic by-products, ending in total disinfection.

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Figure captions

Fig. 1. Logarithmic reduction of 10^6 CFU mL^{-1} *Pseudomonas aeruginosa* with electrolysis time for the electrochemical oxidation (EO) of 100 mL of an aqueous suspension with 7 mM Na_2SO_4 at pH 5.8 using an undivided cell with a boron-doped diamond (BDD), RuO_2-based or IrO_2-based anode and a stainless steel (SS) cathode, all of 3 cm^2 area, at current density (j) of 33.3 mA cm^{-2} and 25 ºC.

Fig. 2. Change of active chlorine concentration with time for the electrolysis of 100 mL of 7 mM Na_2SO_4 + 1 mM NaCl at pH 5.8 in an undivided cell with a: (●) BDD, (▲) RuO_2-based and (■) IrO_2-based anode and a SS cathode at j = 33.3 mA cm^{-2} and 25 ºC.

Fig. 3. Logarithmic reduction vs. electrolysis time for the EO treatment of 100 mL of 10^6 CFU mL^{-1} *Pseudomonas aeruginosa* suspensions with 7 mM Na_2SO_4 + 1 mM NaCl at pH 5.8 in an undivided cell with a BDD, RuO_2-based or IrO_2-based anode and a SS cathode at j = 33.3 mA cm^{-2} and 25 ºC.

Fig. 4. Variation of active chlorine concentration with electrolysis time for the EO treatment of 100 mL of 7 mM Na_2SO_4 with different NaCl contents at pH 5.8 in an undivided BDD/SS tank reactor at several j values and 25 ºC. (a) 7 mM NaCl at j of (■) 8.3 mA cm^{-2} and (●) 33.3 mA cm^{-2}. (b) (▲) 1 mM and (◆) 3 mM NaCl at j = 33.3 mA cm^{-2}.

Fig. 5. Logarithmic reduction of 10^6 CFU mL^{-1} *Pseudomonas aeruginosa* over electrolysis time for the EO of 100 mL of aqueous bacterial suspensions under the conditions of Fig. 4.

Fig. 6. Effect of paracetamol concentration on the logarithmic reduction of *Pseudomonas aeruginosa* with electrolysis time for the EO treatment of 100 mL of aqueous bacterial suspensions with 10^6 CFU mL^{-1} in 7 mM Na_2SO_4 at pH 5.8 using un undivided cell with a: (a) BDD or (b) IrO_2-based anode, and a SS cathode at j = 33.3 mA cm^{-2} and 25 ºC.

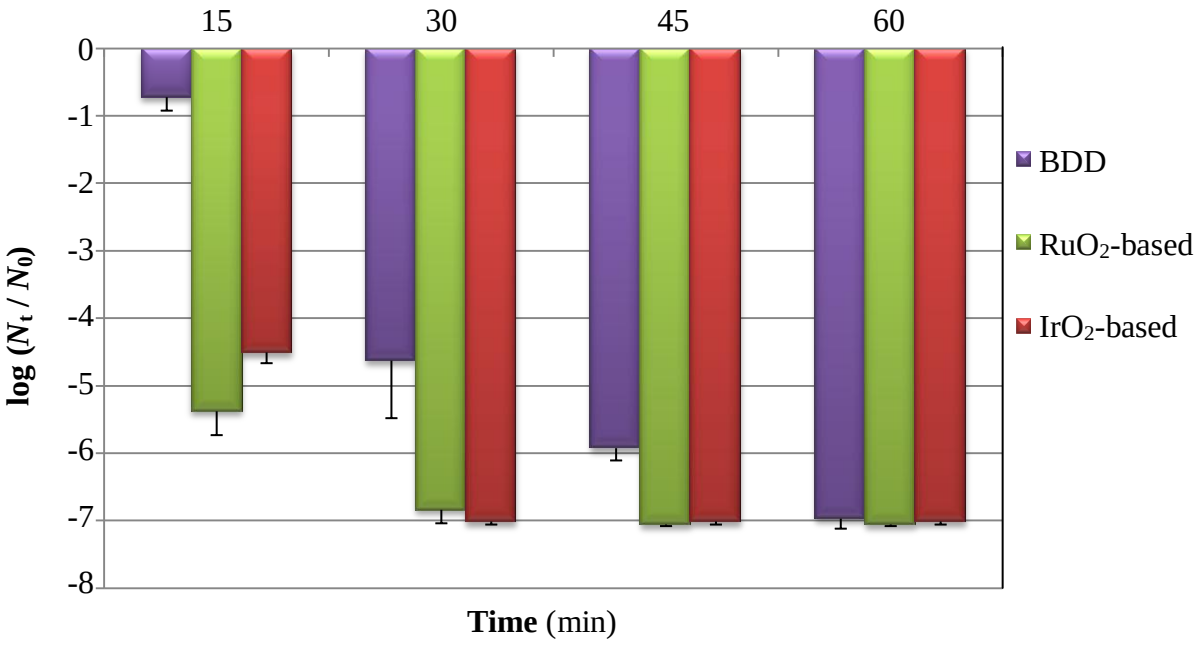

Fig. 1

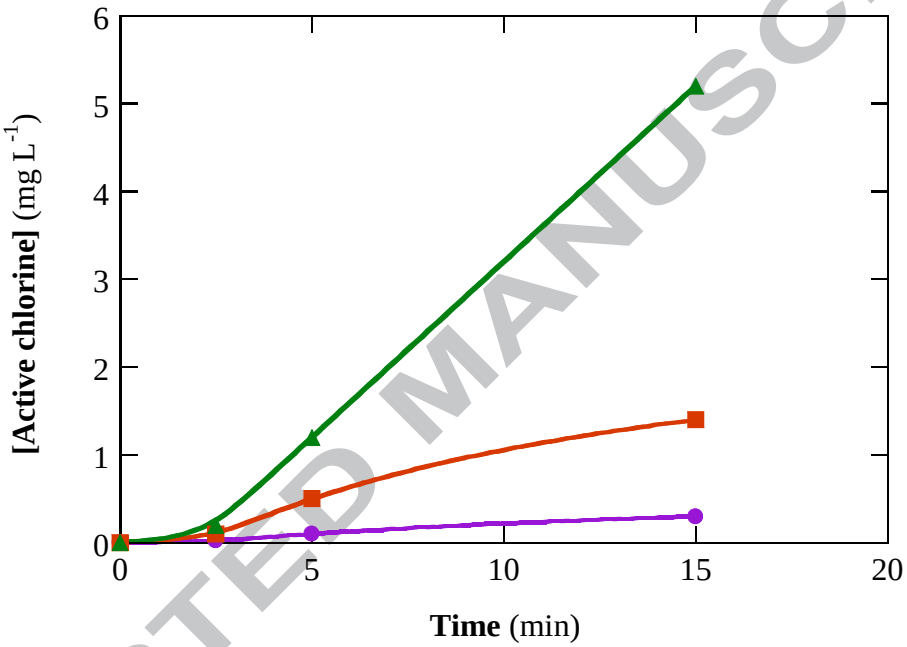

Fig. 2

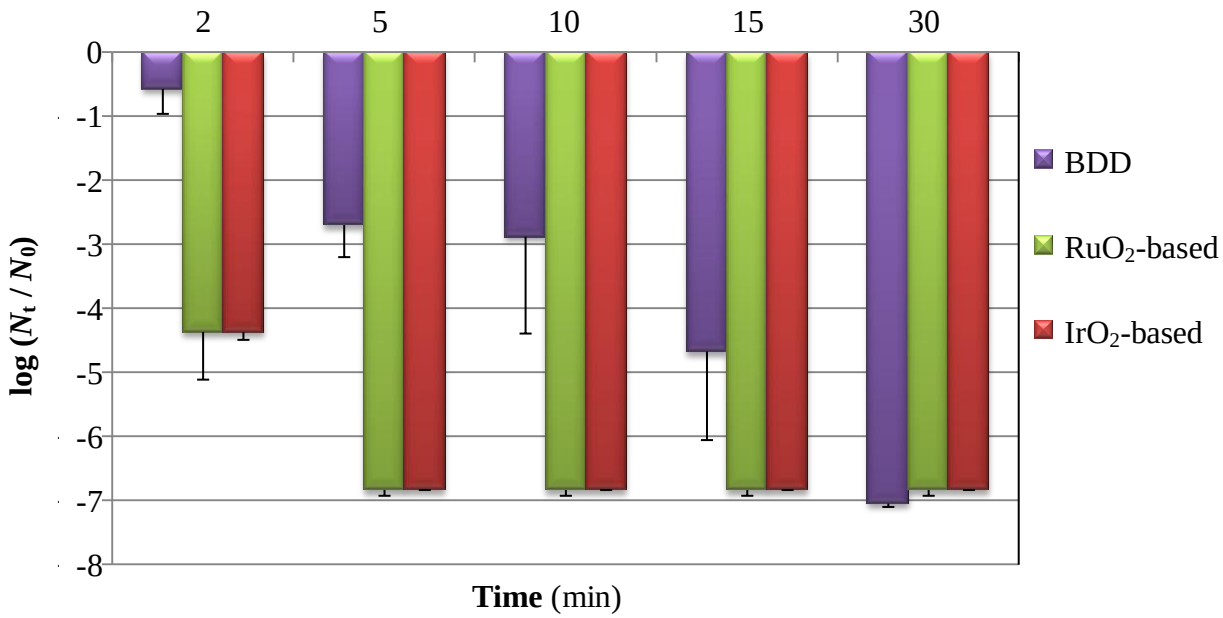

Fig. 3

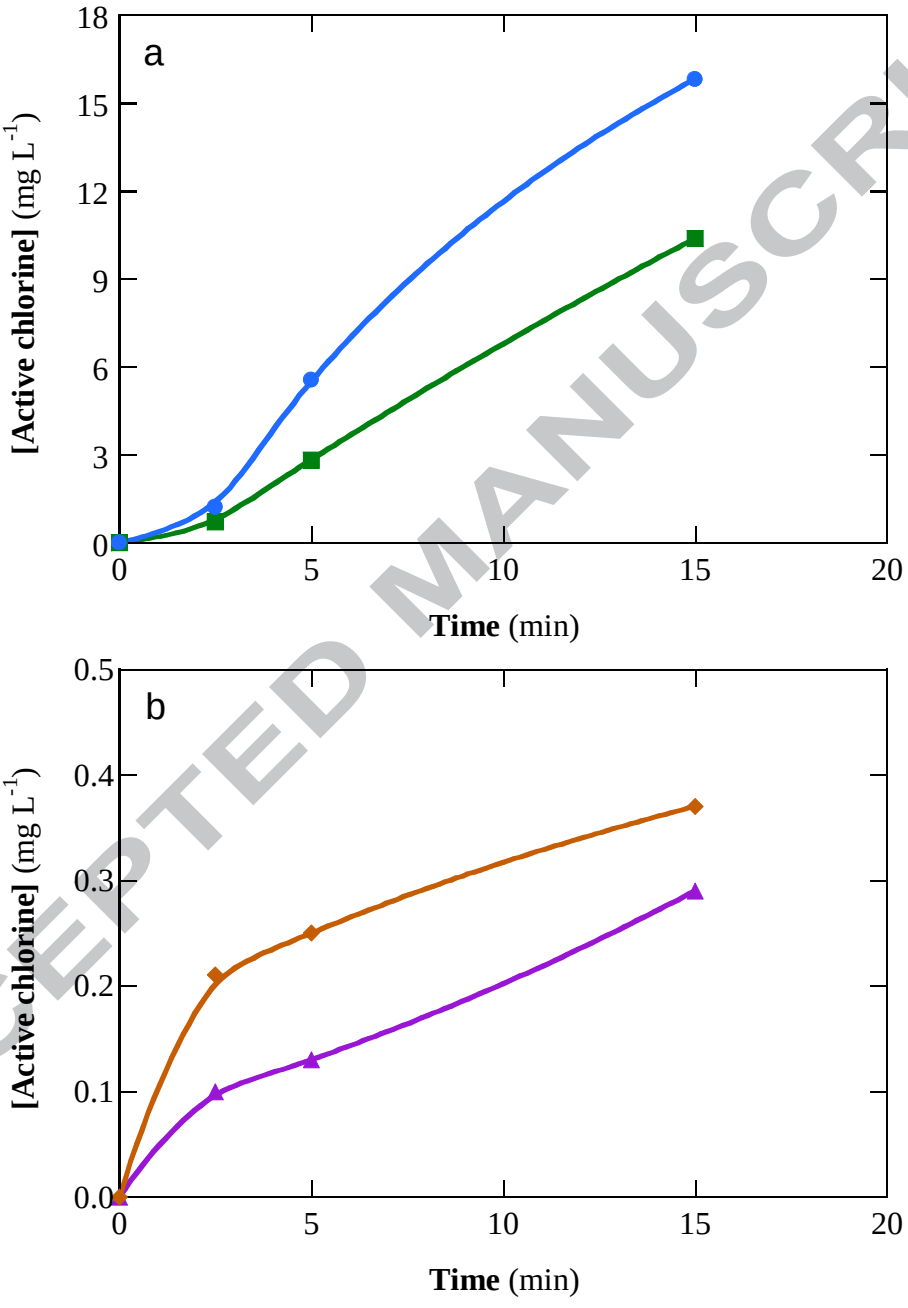

Fig. 4

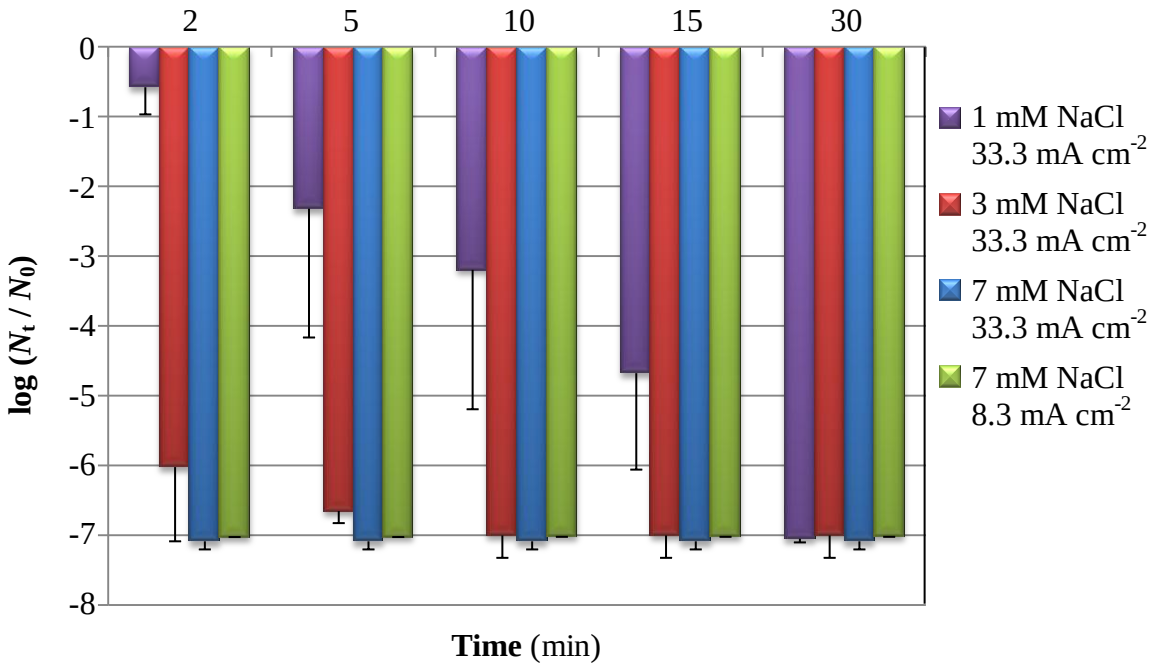

Fig. 5

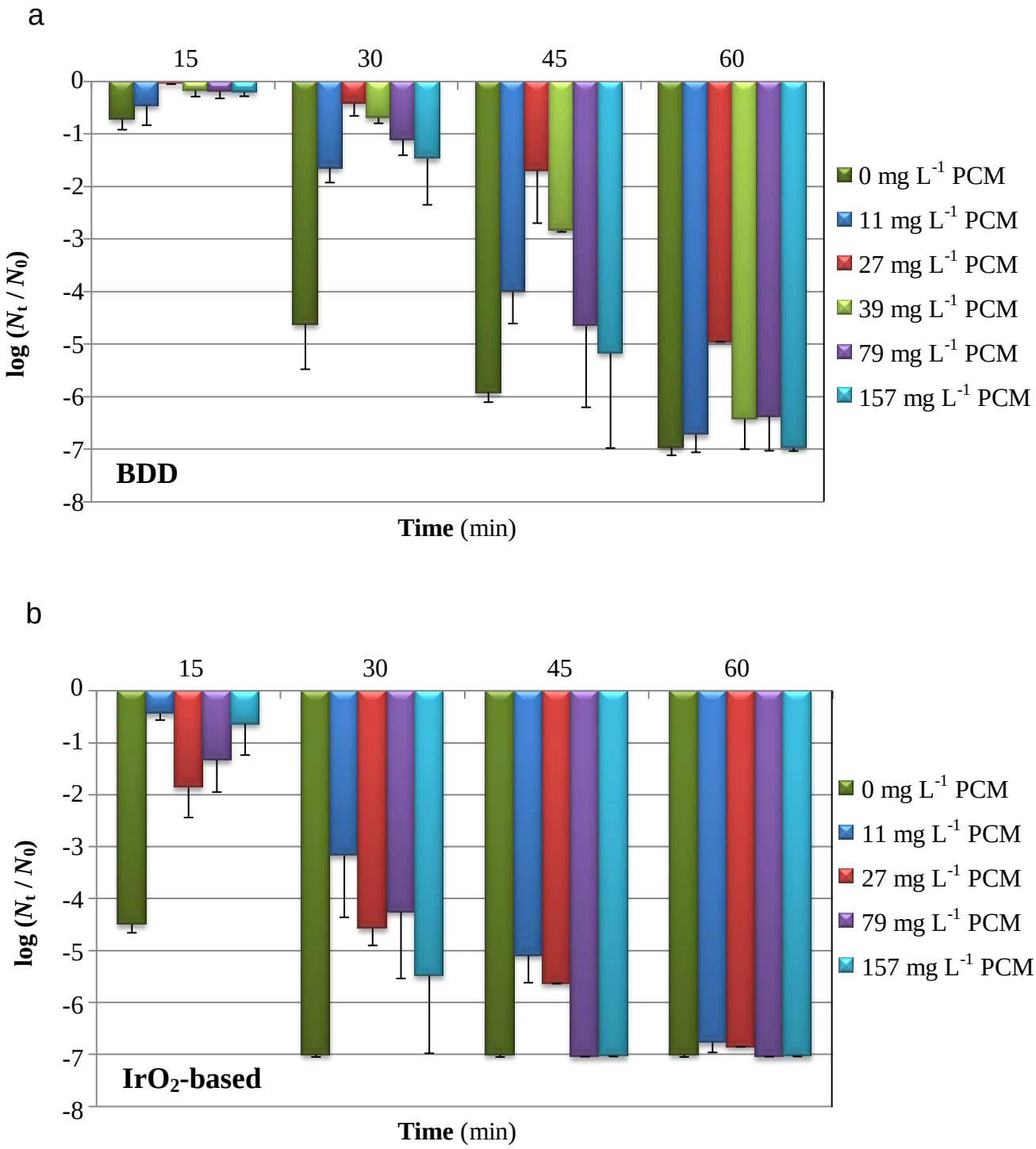

Fig. 6

Research Highlights

- Total inactivation of *Pseudomonas aeruginosa* by electro-oxidation with BDD and DSA
- SO_4^{2-} medium: Faster inactivation using DSA due to the enhanced bacteria adsorption
- Cl^- medium: Faster inactivation using DSA due to the greater active chlorine production
- Paracetamol: surface blockage, $^{\bullet}OH$ scavenger, toxic by-products for Pseudomonas
- Efficient disinfection at low *j* and high Cl^- content, along with gradual decontamination