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EC MICROBIOLOGY
Short Communication

Microorganisms' Electrochemical Disinfection Phenomena

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Received: July 08, 2017; **Published:** July 13, 2017

Abstract

Regardless of the reality that the deactivation of bacteria by different electrochemical (EC) disinfection means has been largely reported in the literature, the influence of process variables and reactor conception on kill performance has not been well comprehended. Moreover, a small number of researches focused on the mechanisms of EC disinfection. This review concentrates on EC microbial killing mechanisms. Several related and pertinent references are examined and key mechanisms are revealed. Some mechanisms have been suggested to interpret the deadliness of EC application, comprising (1) oxidative stress and cell loss of life because of electrochemically produced oxidants, (2) irreversible permeabilization of cell membranes by the placed electric field (EF), (3) EC oxidation of vital cellular constituents during exposure to electric current or induced EFs, and (4) electrosorption of negatively charged microorganisms cells to the anode surface followed by direct electron transfer reaction.

Keywords: *Electrochemical (EC) Disinfection; Electric Field (EF); Boron-Doped Diamond (BDD); Stainless Steel (SS); Advanced Oxidation Process (AOP); Reactive Oxygen Species (ROSs)*

Introduction

Electrochemical (EC) killing of bacteria and yeast cells has been largely reported [1-10]. Some mechanisms have been suggested to interpret the deadliness of EC application, comprising (1) oxidative stress and cell loss of life because of electrochemically produced oxidants, (2) irreversible permeabilization of cell membranes by the placed electric field (EF), (3) and EC oxidation of vital cellular constituents during exposure to electric current or induced EFs [11-26].

Chemical oxidants are produced while electric current is placed on aqueous suspensions of microbes with plunged electrodes [12,27-31]. Electrolysis at the electrodes produces a diversity of oxidants in the presence of oxygen, comprising hydrogen peroxide and ozone, as well as free chlorine and chlorine dioxide when chloride ions are present in the solution [6,12,27,32]. These oxidants are accountable for most, but not all, of the deadliness of the applied direct current (DC) [27]. Some references illustrate that antimicrobial agents and electric current take action interactively to demobilize microbes [6,33-38].

EFs are by their nature poisonous to cells. It has been indicated that this is firstly because of the irreversible permeabilization of the cell membrane [39-41]. Tests performed on artificial bi-film lipid membranes illustrate that a membrane exposed to an outer EF collects charge much like a capacitor, and a transmembrane potential is formed [6]. A short-lived steady-state current through the membrane is initiated when the membrane is completely loaded, illustrating a created permeability of the membrane to hydrophilic molecules. This process is widely entirely interpreted by models implicating the apparition of transient pores in the membrane because of exposure to the outer EF. Two crucial parameters affect the reversibility of this electro-permeabilization: The amount of the created transmembrane potential, and the time of the application of the outer EF. For cells, transmembrane potentials over 1 V and longer pulse times conduct to

irreversible permeabilization and cell dying. The transmembrane potential created by an outer EF relies on the size of the cell membrane, with bigger cells experiencing a bigger transmembrane potential from an applied EF. Consequently, the amount of the field needed to demobilize yeast cells is usually lower than that required to demobilize bacteria [13]. Death happens because of either the apparition of constant pores and following destabilization of the cell membrane or mislaying of crucial cell constituents and demolition of chemical gradients via transport across transient pores [39]. If produced oxidants by EC means are present, these pores can let the oxidants open entrance to the inside of the cell, contributing to the deactivation phenomena [42-45].

EFs have as well the capacity of demolishing cells in the absence of demolishing their membranes. Matsunaga., *et al*. [16] detailed a method in which cells were eliminated in the absence of breaking, only a little with the EC oxidation of intracellular coenzyme A [46,47]. Therefore, EFs can straight oxidize cellular compounds, conducting to cell dying [6,48-50].

Several researches have been centered on the employ of EFs and currents to eliminate bacteria and yeast in industrial and medical applications, as indicated by the next cases. Potable water polluted with *Escherichia coli* (*E. coli*) K12 (100 cells/cm^3) was disinfected at a rate of 600 cells/cm^3/h with the use of a 0.7 V electric potential using a carbon cloth electrode [16]. Potable water polluted with 335 cells/mL total coliforms and 1035 cells/mL fecal streptococci was sterilized with a 2.5 mA/cm^2 DC density (125 mA current) applied with 5 cm × 5 cm titanium electrodes for 30 min [17]. DC (60 mA) was employed to impede the development of *E. coli, Bacillus subtilis, Pseudomonas aeruginosa* (*P. aeruginosa*), and *Staphylococcus aureus* contaminants of a bioprocess reactor [18]. Grahl and Markl [14] performed a study for a non-thermal pasteurization method that may avoid to touch the vitamins, enzymes, texture, and taste of treated foods. They examined the influences of pulsed EFs on *E. coli* and *Saccharomyces cerevisiae* suspended in milk and fruit juice, respectively [14]. Bacteriophages remain alive short applications to different current magnitudes in an EC cell better than bacteria at both low (1×10^3 CFU or PFU/mL) and high (1×10^6 CFU or PFU/mL) population density [6]. Electrolyzed water had been proven to possess an elevated lethal performance than $Ca(OCl)_2$ of the alike measured active chlorine dose [51]. During the treating time, fundamentally internal cell constituents of the microorganisms enter in chemical reactions with the disinfectants [52-57].

Regardless of the reality that the deactivation of bacteria by different EC disinfection means has been reported in the literature, the influence of process variables and reactor conception on kill performance has not been well comprehended. Moreover, a small number of researches focused on the mechanisms of EC disinfection. This review concentrates on EC microbial killing mechanisms. Several related and pertinent references are examined and key mechanisms are revealed.

Influence of electrode material on the microbial inactivation

The type of electrodes has a fundamental contribution in electrocoagulation (EC) process. Ghernaout., *et al*. [1] used ordinary steel, stainless steel (SS), and aluminum electrodes. Ordinary steel (U = 12 V) and aluminum (U = 11.8 V) give to the solution $Fe^{2+}_{(aq)}$ and $Fe^{3+}_{(aq)}$ (neutral pH) and $Al^{3+}_{(aq)}$, respectively; however, SS (U = 10.7 V) does not produce any ions to the solution. Reduction of cellular concentration at 620 nm as a function of electrode nature is shown in Figure 1. For the first 10 min (Figure 1), SS (55.45%) is less efficient than ordinary steel (97.18%), which is less than aluminum (98.16%). These results may be explained by the fact that all bacteria are not eliminated or demobilized; a portion of the bacteria may be absorbed on flocs generated during electrocoagulation using Al or Fe electrodes [30,58].

Jeong., *et al*. [28] studied the action of electrode material on the formation of oxidants, and illustrated the various reaction mechanisms for forming individual oxidants by using boron-doped diamond (BDD), Ti/RuO_2, Ti/IrO_2, $Ti/Pt–IrO_2$, and Pt as anode materials. The performance of ●OH generation was in the arrangement of BDD $\gg Ti/RuO_2 \approx$ Pt. No crucial formation of ●OH was detected at Ti/IrO_2 and $Ti/Pt–IrO_2$. The ●OH was proved to have a fundamental contribution in O_3 formation at BDD, but not at the other electrodes. The generation of active chlorine was in the arrangement of $Ti/IrO_2 > Ti/RuO_; > Ti/Pt–IrO_2 >$ BDD > Pt. The great gap in this arrangement, from that of reactive oxygen species (ROSs), was assigned to the divergence in the electrocatalytic activity of each electrode material with regard to the formation of active chlorine.

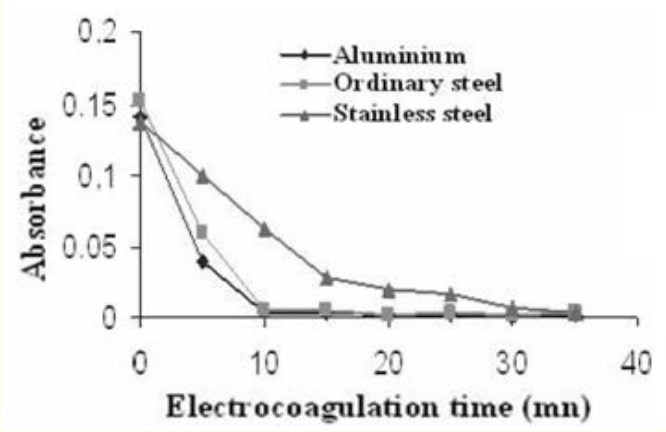

Figure 1: *Reduction of cellular concentration of E. coli as a function of electrodes nature (I = 1 A) [1].*

Similar results were achieved by López-Gálvez., *et al* [8]. Table 1 presents the important mechanisms proposed explaining the deadliness of EC technique and their respective cited references.

Oxidants	EF		
Oxidative stress and cell loss of life [6,20,36,43,46,53].	Irreversible permeabilization of cell membranes [1,5,9,30].	EC oxidation of vital cellular constituents [1,5,47,51].	Electrosorption of negatively charged *E. coli* cells to the anode surface + direct electron transfer reaction [5,29,45,54].

Table 1: *Main mechanisms suggested interpreting the deadliness of EC treatment and their cited references.*

EC control of bacterial persister cells

The appearance of antibiotic-resistant bacteria has given an augmenting defiance to infection monitoring [9]. Classical techniques of antibacterial remediation including elevated dose of antibiotics or surgical intervention have been shown inadequate for eliminating constant infections, such as those linked with medical implants. It is well established that bacterial populations frequently hold a low percentage of phenotypic variants, called persister cells, which are metabolically idle and very resistant to antibiotics. When the antibiotic remediation is ceased, remaining alive persister cells may revive the bacterial population with a comparable percentage of persister cells. Therefore, pertinacity gives a hard defiance to curing chronic infections. Niepa [9] presented a new technique for monitoring bacterial pertinacity founded upon a process which was called EC control of persister cells. Niepa [9] proved that bacterial persister cells may be efficaciously removed by low-level DC; as an example, remediation with 70 $\mu A/cm^2$ DC for 1 h utilizing SS 304 decreased the number of viable planktonic persister cells of *P. aeruginosa* PAO1 by 98% in comparison with the untreated control. Niepa [9] proved that DC applications have an effect on surface charge and membrane integrity of *P. aeruginosa*, conducting to augment intracellular concentration of metal cations. In addition, EC treatments interposed via carbon electrodes induced the permeabilization of the cells to extracellular materials, and elevated their sensibility to antibiotics, which conducted to total elimination of the persisters [59].

Process performance: EC disinfection vs. other techniques

The EC technique was greatly efficient for wastewater remediation [21]. An *E. coli* eliminating performance of 100% may be obtained for the model water with a residence time of only 0.5 min and a current density of 25 mA/cm^2 (Table 2). While the current density was decreased to 16 mA/cm^2, a residence time of 2 min was required to give a disinfection performance of 99.98%. EC disinfection was much more performant than classical chlorination. A residence time of at least 30 min was needed for chlorination to reach a bactericidal

performance of 99.94% or greater. EC disinfection seemed to possess a germicidal performance even bigger than ozonation in terms of residence period. The Fenton reaction was not illustrated as the most efficient disinfection techniques for the model water; however, this was probably formed by the low dosage of Fenton's reagent used in the experimental tests in comparison with the most Fenton reaction conditions.

Disinfection method	Testing conditions	Killing efficiency (%)
EC	16 mA/cm^2, 2 min	99.98
Disinfection	25 mA/cm^2, 0.5 min	100
Chlorination	5 mg/L, 30 min	99.94
	5 mg/L, 60 min	99.98
Ozonation	10 mg/L, 2.5 min	99.9
	10 mg/L, 5 min	100
Fenton reaction	8.5 mg/L H_2O_2, 0.85 mg/L Fe^{2+}, pH 4, 10 min	99.4
	8.5 mg/L H_2O_2, 0.85 mg/L Fe^{2+}, pH 4, 30 min	99.8

Table 2: *Experimental conditions and bactericidal performances of various disinfection techniques [21].*

Diao., *et al.* [21] concluded that all of the disinfection techniques studied in their research (i.e., EC disinfection, chlorination, ozonation and the Fenton reaction), were powerful in eliminating *E. coli* with an initial density of 10^8/mL in the examined wastewater. With an eliminating performance of 99.4% or greater, almost all of the cells in the disinfected samples lost their viability from the viewpoint of being biologically available to incubation (Figure 2).

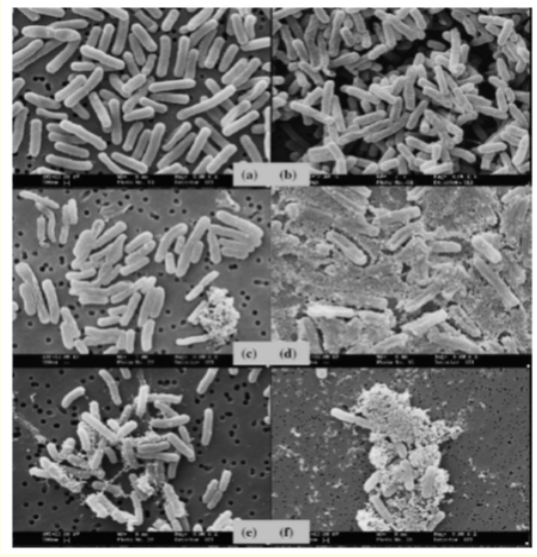

Figure 2: *Scanning electron microscopy (SEM) photographs of E. coli cells in (a) fresh culture and after (b) chlorination at 5 mg/L for 30 min, (c) ozonation at 10 mg/L for 5 min, (d) the Fenton reaction with 8.5 mg/L H2O2 and 0.85 mg/L Fe2+ at pH 4 for 10 min, (e) EC disinfection at 16 mA/cm2 for 2 min and (f) EC disinfection at 25 mA/cm2 for 2 min [21].*

EC disinfection mechanisms

In a general manner, the deactivation of bacteria during disinfection operation may be usually interpreted by two kinds of destruction to bacterial cells [31]. Primarily, disinfectants may enter in reaction with cell area constituents inducing cell membrane permeability vari-

ations or the malfunction of enzymatic diffusion procedures. Secondly, damages to the intracellular constituents, particularly the loss of DNA integrity, may be generated with or without evident cell area destructions [60]. Some disinfectants produce more important deteriorations to either the cell surface area or interior constituents; however, these two kinds of deteriorations are not limited, depending on the *Ct* value (disinfectant dose × residence time) and kind of bacterial cells. While EC disinfection, the behavior of *E. coli* and *Enterococcus* is very various, particularly at the start of the process if the concentration of oxidants was less than 2 mg/L (0-5 min, 4 V) [31]. Comparable findings were observed in the survey on classical chlorination disinfection treatment [61]. The various deactivation kinetics enter the two indicator bacteria are probably linked to their cell surface structure variations (Gram-negative vs. Gram-positive bacteria), because at low chlorine concentration (< 0.5 mg/L, as Cl2), deteriorations of chlorine were detected importantly to the cell areas [62]. When the chlorine dose overpass the minimum (1.5≤ free chlorine ≤3 mg/L), hard deteriorations to bacterial genomes may appear [62,63].

Lacasa., *et a*l. [64] concluded that the main inactivation mechanisms involve (i) mechanical stress (only for *Artemia salina*), (ii) direct oxidation on the surface of conductive diamond anode, and (iii) chemical reactions with chlorine species and/or ROSs (hydroxyl radical, ozone or hydrogen peroxide).

EC disinfection's free radicals: Key contribution in the killing actions

As mentioned previously, the elevated performance of EC disinfection may be given by short-lived and energy rich intermediate products with a more efficient killing capacity [21]. These chemical products obviously comprise free radicals, such as $\bullet OH^-$ and $O_2^{\bullet-}$ [17,65-67]. By their SEM examination (Figure 2), Diao., *et al*. [21] presented more proof of the hypothesis concerning the important contribution of $^{\bullet}OH^-$ radicals in EC disinfection. Cell samples disinfected by $^{\bullet}OH^-$ radicals of the Fenton reaction had a rather comparable look as those after EC remediation. There was crucial degeneration and decomposition of the cells following from both the Fenton reaction and EC disinfection. Liberated cellular materials were collected on the filters, which was remarkable to a lesser amplitude for the samples of ozonation and narrowly remarkable for the samples of chlorination. Consequently, in addition to electro-chlorination, *E. coli* cells during EC remediation were probably deactivated by the intermediate products with an oxidizing strength comparable to that of free radicals and much powerful than that of chlorine [21].

Suggestions of EC disinfection

The real possibilities of the performant disinfection given with the electro-chlorination technique, as an example of EC disinfection, are various. Because disinfection practically may perform with a single pass, the application of the process is very simple in comparison with chemical oxygen demand (COD) elimination, in which recirculation or some type of cascade procedure with several cells has to be utilized. Experience has practically been reached utilizing in-line electro-chlorination for remediation and disinfection of salt water swimming pools, in which the ameliorated disinfection performance from the passing of the cells lets it easy to function at much lower residual free chlorine concentrations (< 0.2 mg/L) that again reduces the concentrations of disinfection by-products (DBPs) [42,68].

Tanaka., *et al.* [48] suggested an EC disinfection system employing a honeycombed platinum coated titanium electrode for the disinfection of seawater. Cell suspensions of the fish pathogens, *Vibrio alginolyticus, Edwardsiella tarda, Lactococcus garvieae* and *Vibrio anguillarum* were circulated in a reactor provided with 10 sets of these electrodes at a flow rate of 200 mL/min with an applied potential of 1.0 V vs. Ag/AgCl reference electrode. The circulated cells were totally killed after 3 h of treatment, whereas free residual chlorine generated due to seawater electrolysis was below 0.1 mg/L. Moreover, a diphenyl-1-pyrenylphosphine fluorescent assay showed that lipid peroxidation in the cell membranes of disinfected bacteria was induced probably by ROSs produced in the course of EC application.

DBPs formation: An EC disinfection undesirable side-effect

As mentioned in the previous Section, chemical water treatment issues such as DBPs formation have incited on the search of better water treatment means such as EC water processes which have been tested with large success in various water/wastewater pollutant treatments. However, their large use is blocked by many technical issues such as chlorine by-products (CBPs) produced species [7]. In fact, in the course of EC treatment, these carcinogenic products may be generated following the electrode material and applied voltage.

In our previous review paper [3], we have discussed the dependence of CBPs produced species generation of the electrode material and applied charge in the course of EC treatment. It was deduced that the usage of electrodes generating highly reactive species has to be more cautiously monitored in hygienically and environmentally oriented using. Following this orientation, Pt and BDD anodes are proved more appropriate than other electrodes. In fact, the good capacity of a BDD anode to generate ROSs and other oxidizing species during the electrolysis allows establishing a chlorine-free disinfection process.

Bergmann and Koparal [7] concluded that practical set-ups must be conceived and monitored in a sophisticated manner. The actual state of non-monitored use of disinfection devices is not favorable in terms of hygienic and health risks considerations. Great works still remain to be performed.

Conclusion

The main important points drawn from this review may be drawn as:

1. EC disinfection has known astonishing focus as an option for classical potable water treatment because of its elevated performance and environmental harmony. The most frequent technique of EC disinfection is the usage of electro-generated oxidants, such as active chlorine and ROSs, as disinfectants.
2. Some mechanisms have been suggested to interpret the deadliness of EC application, comprising (1) oxidative stress and cell loss of life because of electrochemically produced oxidants, (2) irreversible permeabilization of cell membranes by the placed EF, (3) EC oxidation of vital cellular constituents during exposure to electric current or induced EFs, and (4) electrosorption of negatively charged *E. coli* cells to the anode surface followed by direct electron transfer reaction.
3. EFs are by their nature poisonous to cells.

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Volume 9 Issue 4 July 2017