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A THIN LAYER ELECTROCHEMICAL CELL FOR DISINFECTION OF WATER CONTAMINATED WITH *STAPHYLOCOCCUS AUREUS*

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ABSTRACT

A thin layer electrochemical cell was tested and developed for disinfection treatment of water artificially contaminated with *Staphylococcus aureus*. Electrolysis was performed with a low-voltage DC power source applying current densities of 75 mA cm^{-2} (3 A) or 25 mA cm^{-2} (1 A). A dimensionally stable anode (DSA) of titanium coated with an oxide layer of 70%TiO_2 plus 30%RuO_2 (w/w) and a 3 mm from a stainless-steel 304 cathode was used in the thin layer cell. The experiments were carried out using a bacteria suspension containing 0.08 M sodium sulphate with chloride-free to determine the bacterial inactivation efficacy of the thin layer cell without the generation of chlorine. The chlorine can promote the formation of trihalomethanes (THM) that are carcinogenic. *S. aureus* inactivation increased with electrolysis time and lower flow rate. The flow rates used were 200 or 500 L h^{-1}. At 500 L h^{-1} and 75 mA cm^{-2} the inactivation after 60 min was about three logs of decreasing for colony forming units by mL. However, 100% inactivation for *S. aureus* was observed at 5.6 V and 75 mA cm^{-2} after 30 min. Thus, significant disinfection levels can be achieved without adding oxidant substances or generation of chlorine in the water.

Key words: *S. aureus,* disinfection, treatment of water, thin layer cell

INTRODUCTION

The drinking water chlorination may affect its taste and scent, generating hazardous oxidation by-products during treatment, mainly chloramines and trihalomethanes (THM), which are mutagens and carcinogens in organic matter presence (11). Basically, electrochemical oxidation can be achieved through the hydroxyl radical ($OH^{\bullet}$) formed by water discharge in dimensionally stable anodes (DSA). The "reactive oxygen" derived from the hydroxyl radical can

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oxidize microorganisms (7). Also, discharge of water in unbuffered suspension plays an important role in killing microorganisms due to the pH gradient near the electrode (16).

Various electrode materials have been tested for electrochemical water disinfection, focusing on the applied potentials and killing mechanisms. Experiments comparing the performance of anodic materials have been carried out with oxide electrodes (13, 2), activated carbon fiber (12), platinum-clad niobium mesh (10), palladium-coated carbon cloth (19), doped diamond (17), etc. However, most of these materials are only conventional for scientific purposes, but not available in large-scale or economically appealing engineering applications.

Likewise, other methods related to electrolysis or electrical current effects have also appeared, such as disinfection using metal ions generated by electrolytic processes (9), electric inactivation of bacteria in sea water and saline wastewater (18) and electrolytic generation of biocides (22).

Recently, oxide-coated electrodes are used in many studies of electrochemical treatment of water containing organic pollutants. These electrodes and are known as Dimensionally Stable Anodes (DSA) (8) as they are more durable and low cost to maintain. The use of DSA in large-scale water-treatment systems is favored by the ready accessibility of this technology from the chlorine-alkali industry, in which they are employed in electrolyzers (14).

Electrolytic treatment using DSA can improve water disinfection because the addition of a large amount of chemicals is not necessary. The electrolytic treatment is easy to automate, multipurpose, requires only a small area of treatment plant and can cost little to operate. Besides the advantages to public health of not requiring the addition of potentially toxic chemicals, it is an especially clean process, since the electron is the main reactant. Also, it is an efficient method for the removal of pathogens and can be used for disinfection in food processing (4, 6) and medical applications (21). Diao *et al*. (5) tested *Escherichia coli* disinfection by various treatments, including electrochemical disinfection, chlorination, ozonation and Fenton reaction. Scanning electron microscopy analysis suggested that the electrochemical treatment had a greater effect than the other disinfection processes examined.

This paper is based on the design and testing of a thin layer electrochemical cell for the treatment of artificially contaminated water with *Staphylococcus aureus*. Although the bactericidal effect by adding chlorine is well known, the goal in this paper was to study the electrochemical effects, such as the bacterial inactivation in the absence of chlorine compounds.

METHODS

The Gram-positive bacteria *S. aureus* (ATCC6538) were used as a model for disinfection studies. *S. aureus* is a well-known infectious agent. Bacteria cells were cultured aerobically in brain-heart infusion (BHI) at 35°C for 24 h and after that kept in refrigerator until 7 days. Before tests, a 1.00 mL aliquot of cultured cells in BHI was transferred to 100 mL of nutrient broth (beef extract 3.00 g; peptone 10.00 g, sodium chloride 1.50 g diluted in 1000 mL of deionized water). Thus, the culture was shaken in a BOD incubator at 28°C for 24 h. Aliquots of 15.0 mL of this culture were centrifuged at 1.66 “g” for 10 min, washed in deionized water containing 0.08M Na_2SO_4 and resuspended in 15.0 mL of Na_2SO_4 solution, to give the inoculum’s suspension, consisted of young cells free of chloride, for disinfection tests.

The concentration of *S. aureus* suspension was

determined by colony forming units (c.f.u.) by mL using the inoculum's suspension diluted in 3.0 L of 0.08M Na_2SO_4 that results c.f.u. close to 10^6 cells mL^{-1} that was used at the reservoir during the electrochemical treatment. The samples from the reservoir at the electrochemical system (Figure 1) at different times during the electrochemical treatment operated by batch recirculation mode were diluted and spread uniformly on a nutrient agar plate. After 72 h incubation at 37°C, the colony forming units (c.f.u.) were counted and the number of bacteria was calculated for the suspension before and after treatment. In all counts at least three replicate plates were used.

A schematic diagram of the treatment system is shown in Figure 1. It consists of a thin layer electrochemical cell, a hydraulic pump to circulate the solution, a flow meter, a 5.0 L reservoir, pipes and valves. The components of the thin layer electrochemical cell are housed in a metal structure of dimensions 0.70 m x 0.40 m x 1.00 m (l x w x h). The apparatus setup was operated in batch recirculation mode and all experimental runs were carried out with 3 L of bacteria suspension, i.e., *S. aureus*.

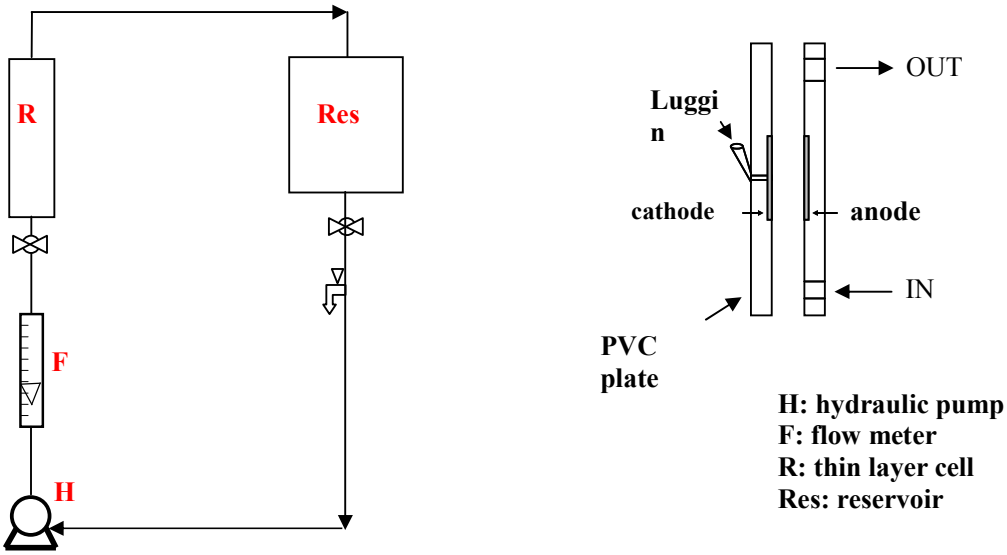

Figure 1: Schematic diagram of the electrochemical system and illustrative view of the thin layer electrochemical cell (right side).

Electrolysis was performed with a low-voltage DC power source (Dawer FCC - 3005D) applying 25 mA cm^{-2} (1 A) or 75 mA cm^{-2} (3 A) at 5.6 V. As anode was used a DSA rectangular electrode of titanium (reaction area 40 cm^2), coated with an oxide layer of 70%TiO_2 and 30%RuO_2 (w/w) and a stainless-steel 304 cathode (3 mm from anode) installed inside the single-compartment of thin layer cell made of PVC plates. The DSA is designed for high mechanical strength, high physical and chemical stability over a wide pH-range and insolubility in aqueous media, so that it has a long

operational life and is not toxic; it also exhibits a high overpotential for oxygen evolution (8, 14).

Electrolysis experiments were carried out in suspension with chloride-free 0.08 M sodium sulphate, to determine the bacterial inactivation efficacy of the thin layer cell without the generation of chlorine or adding chemical oxidants. The flow rates were varied (200 or 500 L h^{-1}). All reagents were P.A. grade and also a Digimed DMPH-2 pHmeter and a Tecnopom CA150 conductivimeter were used for physical-chemical analysis.

RESULTS AND DISCUSSION

The effects of electrolysis on the survival rate of *S. aureus* (at a flow rate of 200 and 500 L h^{-1}) are shown in Figure 2. As expected, electrolysis plays an important role in the final viability of the cells. *S. aureus* inactivation increased significantly through electrolysis time, current density and lower flow rate. Thus, 100% inactivation for *S. aureus* was observed after 30 min at a potential of 5.6 V, current density of 75 mA cm^{-2} and 200 L h^{-1}. However, the Figure 2 shows an intermediary condition with 500 L h^{-1} and 75 mA cm^{-2}. In this case, the *S. aureus* inactivation decrease was lower than three logs after 60 min, demonstrating that significant disinfection levels can only be achieved at 200 L h^{-1} and 75 mA cm^{-2}.

Furthermore, the low power consumption needed to achieve these levels (zero c.f.u. at 200 L h^{-1} and 75 mA cm^{-2}) is 8.4 kWh m^{-3}, which is a notable feature of this electrolytic treatment. Moreover, the inactivation of *S. aureus* was not caused by chlorine, since the electrolytic medium was absent of chloride ions, which generates chlorine by electrolysis. Consequently, the bactericidal agent under these conditions is believed to be the physiosorbed hydroxyl radicals between electrode and microorganisms. The hydroxyl radicals are formed, as proposed by Comninellis and Platner (3), during the electrolysis of water by anodic catalysis (equation 1):

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

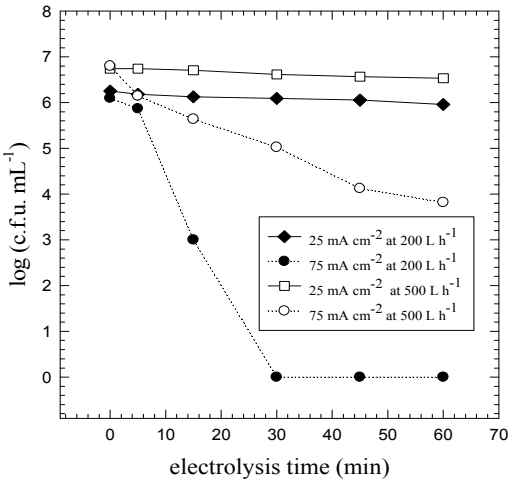

Figure 2: Logarithm of colony forming units (c.f.u.) in function of electrolysis time for different electrical currents and flow-rates.

Thus, increasing the current density increases the rate of $OH^{\bullet}$ generation that is extremely reactive and, also increases the H^+ in the vicinity of the anode leading a greater disinfection (Figure 2). The proposal mechanism is that the microorganisms come into contact with the anode surface, get adsorbed onto it, and be oxidized by $OH^{\bullet}$. This can be interpreted as an electrooxidation, such as electrochemical oxidation of coenzyme A (15) or destabilization of the cell by the transfer of charge from $OH^{\bullet}$ (7). However, cellular lyses due to the electroporation in the cell membrane due to high electric fields at electrode surface cause damage to the cells (20). In addition to this, low pH values nearby the electrode

surface due to the formation of H^+ on anode during the anodic discharge of water turns unviable the cell machinery.

In the course of these experiments, there was no significant heating due to electrolysis. The temperature increased 5ºC at most; also, the pH value observed at suspension was around 7.0 and the conductivity was practically unaltered. Since the solutions did not exhibit any significant rise in temperature or pH change at bacteria suspension during the electrolysis, pH of suspension, temperature and conductivity were not responsible for the bacterial inactivation. The data for 75 mA cm^{-2} at 200 L h^{-1} (Figure 2) leads to a first order kinetic as described by equation 2:

$$\text{Log}(N_o/N) = k.t \qquad (2)$$

where No is the initial bacteria cell population that is 1.25 x 10^6, N is 10^{-6} cell to be considered a sterile assurance level (SAL) according to Allison (1), k is the velocity constant and is equal to 0.21 min^{-1} thus t is 58 min to reach a total sterile suspension.

CONCLUSION

The present study shows that it is possible to obtain a bactericidal effect without generation of chlorine or oxidant compounds. The results infer that is possible to extend the concept for larger-scale disinfection by a thin layer electrochemical cell by simply increasing the number of electrode cassettes and maintaining the power consumption rate. Considering the number of bacteria cells inactivated by the electrolytic process, it becomes efficient for reducing the numbers of potentially pathogenic bacteria and ensuring the safety of the treated water. However the proposed system could not be applied as unique treatment due to legal regulations, as it would be better a combination with other methods, such as UV disinfection for tertiary treatment of potable water. Treated water without chlorine or oxidants compounds can improve human health due to the deleterious effects caused by chemical oxidants in the water.

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RESUMO

Uma Célula Eletroquímica de Camada Delgada para Desinfecção de Água Contaminada com *Staphylococcus aureus*

Uma célula eletroquímica de camada delgada foi utilizada e desenvolvida para a desinfecção de água contaminada artificialmente com *Staphylococcus aureus*. A eletrólise foi executada com uma fonte de corrente direta utilizando 75 mA cm^{-2} (3 A) ou 25 mA cm^{-2} (1 A). Um anodo dimensionalmente estável (DSA) de titânio revestido com uma camada do óxido de 70%TiO_2 e 30%RuO_2 (w/w) e distanciado por 3 milímetros de um catodo de aço inoxidável 304 foi utilizado para gerar uma camada delgada de suspensão bacteriana passando pela célula de camada delgada. As suspensões utilizadas eram feitas apenas com Na_2SO_4 0,08 M e livre de íons cloretos de forma a inativar as células bacterianas no tratamento eletroquímico sem a geração de cloro, este pode promover a formação dos trialometanos (THM). As taxas de fluxo em recirculação foram 200 ou 500 L h^{-1}. A inativação do *S. aureus* aumentou com o tempo de eletrólise e a uma taxa de fluxo menor. Assim, a inativação de 100% para o *S. aureus* foi observada após 30 min a 5,6 V e 75 mA cm^{-2} . Em 500 L h^{-1} e 75 mA cm^{-2} a inativação decresceu em três logs de unidades

formadoras de colônias por mL após 60 min. O tratamento eletroquímico utilizando uma camada delgada promove a desinfecção completa de *S. aureus* sem a necessidade de adicionar substâncias oxidantes ou a geração de cloro.

Palavras-chave: *S. aureus*, desinfecção, tratamento de águas, célula de camada delgada

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