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Elimination of pathogenic bacteria using electrochemical process containing steel mesh electrode

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ABSTRACT

The main objective of this study is the removal of pathogenic bacteria using electrochemical process. The bactericidal effects of electrochemical system containing steel mesh electrode were evaluated from contaminated water with *Escherichia coliPseudomonas aeroginosa, Staphylococcus aureous* and *Streptococcus fecalis* at various concentrations. Effect of current density, supporting electrolyte and pathogenic bacterial concentration were studied and kinetic rate of disinfection were determined. The results obtained show that the effect of current density on disinfection efficiency was highest and the concentration of bacteria in the contaminated water and supporting electrolyte concentrations in the electrolyte were more effective. Contaminated water including 100 and 1000 bacteria/ml of *E. coli, P. aeruginosa, S. aureus* and *S. faecalis*, were completely disinfected using 0.088 mA/cm^2 current in a duration of 5 to 60 min. The rate of the reaction of disinfection is directly related to current density. It is expected that experimental results could be used as a reference for the elimination of pathogenic bacteria using electrochemical process.

Keywords: Disinfection, Kinetic, Bacteria, Electrochemical, Water, Pollution

Introduction

Chlorination is considered as one of the most common and efficient method for disinfection of contaminated water. Nevertheless, it has been reported that the reaction of chlorine with organic compounds which exist in water caused the production of some byproducts like trihalomethanes, haloaceticacids that could caused some problems in the removal of resistant pathogens[1,2]. Because of the carcinogenic and mutation characterization of chlorine byproducts, they constitute a major problem of chlorinated water. Moreover, the mentioned compounds cause some changes in taste and odor of water.[3] In general, microbial agents, if exposed to unfavorable conditions such as changes in osmotic pressure, electrical current, heat, cold, chemical substances, antioxidant and ultraviolet rays, could changed and these factors can be used in disinfection of water. According to this mechanism of action, various methods have been proposed for disinfection of water such as reverse osmosis, coagulation and flocculation processes, ultraviolet light, ultrasonic radiation, ozone, hydrogen peroxide, photolysis and photo-catalysis, radiolysis, electro-hydrolytic discharge, high voltage systems, and electrolysis.[4,5] The research conducted have shown that some of the treatment methods, such as chlorination, do not have adequate effectiveness against some resistant pathogens. Pathogens such as *Giardia lamblia*, *Cryptosporidium parvum*, some gastrointestinal viruses and microsporidian are among these microbial agents. Between the various proposed methods, disinfection by electrochemistry is a

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practical and efficient technique as alternative for chlorination.[6]

Massoudinejad et al., obtained 96.36% efficiency for disinfected water contaminated with *Escherichia coli* using electrochemistry method.[7] Rezaee et al examined the elimination of *E. coli* from contaminated water using electrolysis method. The system could removed 10^2 and 10^3 bacteria per milliliter sample with more than 97% efficiency.[8] The electrochemical disinfection of *E. coli* were studied in the presence of a supporting electrolyte at a concentration of 500 mg/l. The results reveal that the electrochemical disinfection method was more effective than the other methods. [9]

The electrochemical process has been reported to be very successful in solving of pollution problems and they could enhance the quality of physico-chemical and biological water, industrial and domestic wastewater.

Mechanism of electrochemical disinfection have been reported as follows: direct oxidation on the surface of the anode and indirect oxidation in the solution. This technology can be used to reduce organic compounds and water disinfection without the presence of chloride. Therefore, it can operate without the formation of toxic chlorine byproducts.[10]

Electrochemical disinfection process is a simple, efficient and low cost process, with no need to add chemical substances such as chlorine, and require low space to operate. In the electrochemical process, high current density led to the dissolution and corrosion of anode electrode in water. Between the proposed electrodes, stainless steel is a hard, strong, durable, malleable alloy of iron and carbon and resistant to water damage and corrosion. In addition, the specific mesh area is higher in comparison to sheet.

To the best of our knowledge and based on the literature, there is no previous report on the electrochemical removal of pathogenic bacteria using stainless steel mesh electrodes. Hence, in this study, steel mesh electrodes and less current intensity were employed for the removal of pathogenic bacteria. The main objective of this study was to determine the rate of removal of pathogenic bacteria from contaminated water using the electrochemical system with stainless mesh electrode. Moreover, the effect of operating parameters such as induced current, nature of anode, supporting electrolyte in the removal of pathogenic bacteria from contaminated water were evaluated.

Materials and Methods

Preparation of pathogenic bacteria

In this laboratory study, *E. coli* and *Pseudomonas aeruginosa*, as pathogenic gram-negative bacteria and *Streptococcus faecalis* and *Staphylococcus aureus*, as pathogenic gram-positive bacteria were prepared from the Ministry of Health Reference Laboratory.

To investigate the elimination of pathogenic bacteria by electrochemical system, the initial concentration of bacteria (10^2 and 10^3 per milliliter) were prepared using McFarland tubes according to the National Committee of standardization of clinical laboratory recommended method.[11] Generally, McFarland solution contains sulfuric acid 1% and barium chloride dehydrate ($BaCl_2.2H_2O$) 175.1%. A 0.5 McFarland Standard tube represents 1.5×10^8 of bacteria per milliliters. For preparation of 0.5 McFarland tube, 0.05 and 9.95 ml of barium chloride solution and sulfuric acid were mixed in water, respectively.[12] Barium chloride creates milky color in the presence of sulfate and the color intensity depends on concentrations of barium chloride and sulfate. In this method, the optical densities of McFarland tubes could be used for determination of the number of bacteria in the prepared solutions. For preparation of some bacterial concentration, the bacterial solution was diluted with phosphate buffer saline.

Experimental set up

The laboratory experiments were performed in a single electrochemical reactor. The stainless steel mesh were used as anode and cathode electrodes. Electrochemical disinfection was run in a batch mode with a 1000 mL capacity Pyrex glass at ambient temperature (25ºC). The electrodes were connected to a DC Power supply (Atten APS 3005 S-3, China) with on/off operational switches for controlling the constant current density and voltage. Removal of pathogenic bacteria was examined as follows: the number of bacteria for each of the pathogenic bacteria (100 and1000 number of bacteria per milliliter), supporting electrolyte (5

- 20 mg/L NaCl), current densities (0.022 to 0.088 mA/cm^2) and time (5-60 min). The characterization of used urban water, TDS, TSS , EC, pH and turbidity were 349 mg/l,10 mg/l, 518 µs/cm, 7.6 and 0.1 NTU, respectively. For removal of chlorine from urban water samples, one drop of 3% sodium thiosulfate was added to the experimental prepared solutions. During laboratory experiments, samples were taken at 10 min interval and inoculated on nutrient agar. All experiments were carried out in triplicate. The growth colonies on the surface of the nutrient agar media were examined for morphology and biochemical characterization, to ensure the purity of the bacteria studied and lack of contamination in all experiments. The removal efficiency of bacteria was calculated from Equation (1):[13]

Removal (%) = $(1-B_t / B_{t0}) \times 100$ (1)

Where B_{t0} and B_t are numbers of bacteria at time zero and after electrochemical disinfection and t is time of reaction. After each experimental run, the electrodes were washed with distilled water.

Results and Discussion

Chlorination method is a wide and efficient technique applied for disinfection of polluted water. Nevertheless, chlorination is not effective enough to remove some of the pathogenic agents. Therefore, novel processes such as electrochemical process that have more potency for the removal of pathogenic agent have been proposed. Electrochemical disinfection is a high efficient process for bacteria removal from water and wastewater. The results of electrochemical disinfection of water contaminated with pathogenic bacteria (*E. coli*, *P. aeruginosa*, *S. faecalis* and *S. aureus*) in different conditions is shown in Fig. 2a-d. Two-way analysis of variance showed that examined bacteria need different time for removal in the electrochemical disinfection system ($P < 0.05$). The obtained results revealed that at current density of 0.088 mA/cm^2, with initial concentration of 10^2 and 10^3 per ml of examined bacteria, *E. coli* during 5 min, *S. aureus* during 30 min, *P. aeruginosa* during 60 min and *S. faecalis* during 60 min were completely eliminated from the contaminated water. Culture of disinfected water did not grow any bacteria after the electrochemical disinfection.

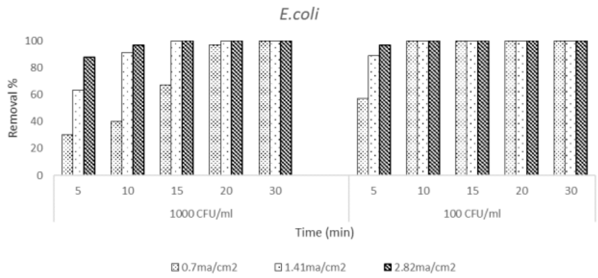

Fig.2a Percentage of live bacteria after electrochemical disinfection containing steel mesh electrode *Escherichia coli*

The percentage of bacteria in the culture medium are presented in Table 1.

To determine the kinetics of electrochemical disinfection process, log (N/No) over time in minutes at different current densities were plotted.

$$\text{Log}(N_t / N_0) = -Kt \quad (2)$$

Where N_0 and N_t are numbers of bacteria at time zero and after electrochemical disinfection and t is time of reaction.

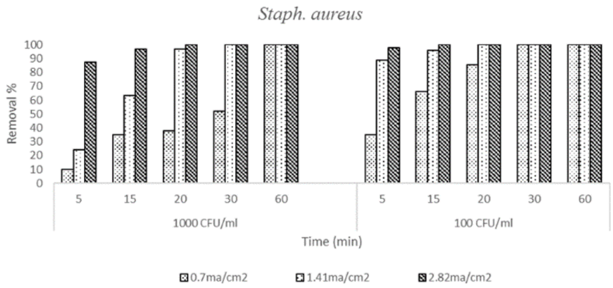

Fig.2b Percentage of live bacteria after electrochemical disinfection containing steel mesh electrode *Staphylococcus aureus*

The slope of the line is represented as K (fixed kinetic inactivation) which means that k shows inactivation rate.[14] Chart of bacterial inactivation usually follows kinetics relationship from first order. The results of electrochemical disinfection kinetics of examined bacteria; *E. coli, S.aureus, P.aeruginosa and S. faecalis* at different current densities are presented in Table 2. The electrical energy consumption is calculated as follows:[15]

$$E = \frac{U.I.t}{V} \quad (3)$$

Where E is energy consumption (kWh/ m^3), U is voltage (V), I is current density (A), t is the time of electrolysis (h) and V is the volume of water (L). The results of energy consumption obtained are presented in Fig. 3.

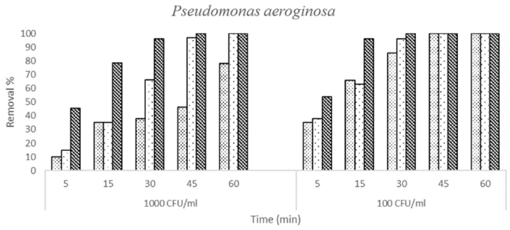

Fig.2c Percentage of live bacteria after electrochemical disinfection containing steel mesh electrode *Pseudomonas aeruginosa*

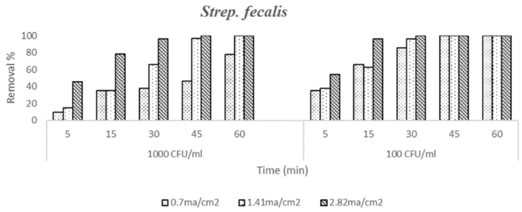

Fig.2c Percentage of live bacteria after electrochemical disinfection containing steel mesh electrode *Streptococcus faecalis*

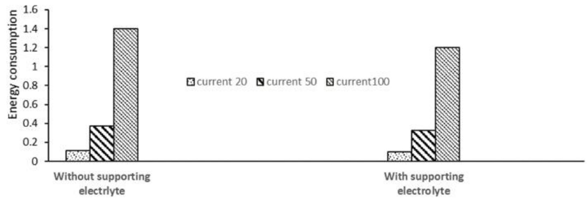

Fig. 3 Comparison energy consumption of the electrochemical reactor with current density 20-100 mA

As a general rule, the disinfection process should not cause any change in the quality of drinking water. Hence, we applied special condition in the electrochemical disinfection to obtain high efficacy and low change in quality of water.

Different interpretations exist regarding the mechanisms of inactivation of microbial electrochemical process.

Table 1 Percentage of live bacteria after electrochemical process with different current densities

Bacteria	**Initial Cencentration Of bacteria (cfu/ml)**	**Percentage Of Live Bacteria per ml in 5 to 60 min operating system Electrochemistry**						**Current density mA/cm^2**
		5	**10**	**15**	**20**	**30**	**60**	
E.coli	10^3	0	0	0	0	0	0	0.088
	10^2	0	0	0	0	0	0	
	10^3	23	0	0	0	0	0	0.044
	10^2	0	0	0	0	0	0	
	10^3	43	22	0	0	0	0	0.022
	10^2	22	0	0	0	0	0	
Staphylococcus aureous	10^3	52	35	12	5	0	0	0.088
	10^2	35	10	0	0	0	0	
	10^3	70	45	2	17	4	0	0.044
	10^2	8	42	2	0	0	0	
	10^3	92	56	35	24	9	0	0.022
	10^2	63	35	19	7	0	0	
Pseudomonas aeroginosa	10^3	70	50	32	19	0	0	0.088
	10^2	35	22	17	4	0	0	
	10^3	85	70	34	25	12	7	0.044
	10^2	40	30	15	7	3	0	
	10^3	90	75	60	50	33	24	0.022
	10^2	45	37	32	29	17	8	
Streptococcus fecalis	10^3	70	52	3	20	10	0	0.088
	10^2	36	24	14	6	0	0	
	10^3	85	74	55	33	17	6	0.044
	10^2	42	33	18	9	2	0	
	10^3	90	72	54	48	37	17	0.022
	10^2	48	36	26	19	15	9	

*Zerotable in the Table means not seen.

Most studies suggest that successful electrochemical disinfection is attributed to bactericidal factors such as pH, current density, supporting electrolyte, bacterial concentration, and redox potential.[16,17] When oxidants are produced in the system, they could create strong oxidation potential. They can attack the bacterial cell membrane and destroy the cell wall and membrane integrity or electrolyze molecules in the cell, thereby leading to cell death and destruction of the cell.[18,19] In electrochemical disinfection, oxidation occurs at anode and reduction at the cathode (Equations 4 and 5)

$O^2 + 4H^+ + 4e^- \rightarrow 2H_2O$ (Anode reaction) (4)

$2H_2O + 2e^- \rightarrow H_2 + 2OH^-$ (Cathode reaction) (5)

In the process, active chlorine such as Cl_2, HOCl , OCl^- and ClO_2 are produced for oxidation of inactive microbial cells. The chlorine compounds in the anode are produced as follows:[20]

$2Cl^- \rightarrow Cl_2 + 2e^-$ (7)

$Cl_2 + 2OH^- \rightarrow H_2O + OCl^- + Cl^-$ (8)

$Cl_2 + 4H_2O \rightarrow 2ClO_2 + 8e^-$ (9)

The reason for the different sensitivity of bacteria may be attributed to difference in outer membrane permeability. According to scientific results, permeability of *P. aeruginosa* outer membrane is 12 to 100 times lower than *E. coli*. The low permeability may be is one of the reasons for their resistance to different antibiotics which is the key features of mucous membranes in *P.aeruginosa*.[21] The cell membrane of a microorganism is a critical location for antioxidant action and access high disinfection efficacy, regardless of the type of antioxidant. When the cell wall is damaged, the cytoplasmic cell composition are released outside the cell which leads to the death of the cell. Hydroxyl radicals attack unsaturated poly-phospholipids existing in the membrane lipid and cause serious damage to the cell wall. Oxidation of membrane lipid causes loss of essential functionality and integrity of the cells, such as cellular respiration, which ultimately leads to cell death.[22] The factors involved in microorganisms' inactivation are not only oxidants which are produced during electrolysis, but also direct current induction which caused the death of the microorganism.[23] In this study, in order to develop the electrochemical disinfection, the stainless steel mesh electrodes were considered for electrolysis of water. *E coli* as an indicator bacteria was used in the. experiments for evaluation of disinfection.

Table 2 Obtained results of K for different examined bacteria in the electrochemical disinfection

Bacteria	Current density mA/cm²	Disinfection kenetic (K)	Removal Time (min)
E.coli	0.088	0.6	5
	0.044	0.3	10
	0.022	0.2	15
Staphylococcus aureous	0.088	0.1	30
	0.044	0.05	60
	0.022	0.05	60
Pseudomonas aeroginosa	0.088	0.05	60
	0.044	0.05	60
	0.022	0.035	60
Streptococcus fecalis	0.088	0.05	60
	0.044	0.05	60
	0.022	0.034	60

The main operating parameters were studied including electrical potential, the concentration of sodium chloride as a supporting electrolyte. Utilization of sodium chloride in concentrations of 5 to 20 mg per liter in the electrochemical disinfection experiments demonstrated that it has no significant role in increasing the efficiency of bacteria removal and reduction of removal time. Considerable points in this case were reducing the voltage to supply the desired current density, economical disinfection process and reduce expenses. Therefore, because there was no effect of adding the supporting electrolyte in the removal efficiency, it can be concluded that reactive oxygen species such as hydroxyl radical, ozone and hydrogen peroxide could be impressive. Some research studies have reported the oxidizing agents for disinfection.[23,24] In our study, chlorine and ozone concentration in the reactor was not measured and the effect of these agents require more study. The results showed that after the electrochemical disinfection, the physico-chemical qualities of water such as TDS, TSS, EC, pH and opacity have no significant difference. Due to the use of low current density, sludge produced was negligible. Generally, water disinfection by electrochemical method is considered as a suitable alternative method for water disinfection against bacteria such as *E.coli, S. fecalis, P. aeruginosa* and *S. aureus*. Complete removal of bacteria in optimum condition using stainless steel mesh electrodes were obtained.

Conclusion

The aim of this study was to investigate the effect of electrochemical process using steel mesh as novel electrode for elimination of pathogenic bacteria. According to the results obtained, the electrochemical process using steel mesh electrodes is a better technique for disinfection of contaminated water with pathogenic bacteria.

Acknowledgments

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