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The electrochemical removal of bacteria from drinking water

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ABSTRACT

Effective disinfection is a particular stage of drinking-water treatment and is an important process for the removal of pathogenic microorganisms from the water. Germs resistant to common disinfectants are a major challenge in drinking water treatment across the world. The present study was conducted to compare the electrochemical removal of *Escherichia coli* and spores of *Bacillus subtilis* as indicative and resistant bacteria in drinking water, respectively. A reactor designed with a capacity of 200 cc and containing steel electrodes was selected for the reactions. The number of bacteria (CFU/mL), the electrochemical reaction time (min), the voltage (v), the electric current intensity (mA), ambient temperature of 25°C and natural pH of drinking water (7.4) were fixed as the operating parameters of the study. Based on the findings of this study, after applied voltage, reaction time is the most effective factor in increasing microbial removal efficiency. The optimal reaction time for the removal of *Bacillus subtilis* spores, *Bacillus subtilis* and *E. coli* was 5, 90 and 120 min, respectively. By establishing a potential difference of 4.5 and 8 V in the reactor, the number of *Bacillus subtilis* spores after the expiry of 2 h was 2 CFU/mL and 0 CFU/mL, respectively.

Keywords: Electro chemistry; *Escherichia coli*; Spore; Water disinfection

1. Introduction

The use of contaminated drinking water has a direct effect on general health. According to the WHO, more than 80% of contagious human diseases are caused by contaminated water [1]. Unsafe water is the main cause of children's mortality in developed countries [2]. More than a billion people in the world still do not have access to safe drinking water [3,4]. The control of contamination, refining and reuse of water in drought-affected countries is very evident [5]. Water disinfection is carried out to remove pathogenic microorganisms from the water. Disinfection has a major role in the conservation of water, and the lack of attention to it will have detrimental effects on the health of human societies [1,6]. Water microbial control is very crucial for having healthy drinking water [7,8]. Coliform microorganisms are known as an appropriate indicator of water quality, since their detection in water is easy. There should be no coliforms in treated water; otherwise, treatment is believed to have not been enough or contamination to have somehow been introduced during the treatment [9,10]. Disinfection is necessary for the control of microbial contamination. The effectiveness of disinfection is affected by the physicochemical properties of water, such as pH, total solids and temperature [10–12]. *Bacillus* spores can remain silent and resistant for years, but exposure to factors such as specific nutrients can bring them back to life over a few minutes in the budding process. This process requires a number of spore-specific proteins, most of which are inside the internal spore membrane [13]. *Cryptosporidium parvum* oocyst is one of the sources of waterborne pathogens that is very resistant to conventional disinfection techniques and is now considered a major public health threat and a global challenge in water resource management. Due to the costs and difficulty of the direct testing of *cryptosporidium*, the use of non-patho-

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genic *Bacillus subtilis* spores as a model and an alternative organism for evaluating the inactivation of cryptosporidium in water is highly efficient [14]. Several methods have been introduced for deactivating microorganisms in water [15]. Electrochemical methods are considered a clean treatment method for water disinfection. In the electrochemical method, microorganisms are decomposed by electric current. An oxygen-based disinfection process, preventing the production of harmful side products, a reliable disinfection of drinking water and pool water and are among the benefits of electrochemical water disinfection. Electric current leads to the production of a variety of disinfectants in the water by electrochemical methods [2,16]. The killing or inactivation of bacteria and fungal cells by electrochemical methods is well documented [9]. The electrochemical process is one of the most powerful methods for the removal of organic contaminants that has received a lot of attention due to its low environmental impact, ease of use and low relative costs. Electrochemical (EC) methods have also been used as an effective process for the treatment of industrial and domestic wastewater [17,18]. This technology can be used to mineralize organic matters and disinfect water even in the absence of chloride and without the production of chlorine and its derivatives. Another advantage of electrochemical disinfection is the decomposition of bacterial cell walls in a way that they cannot be repaired, which is much more severe than the damages caused by the addition of chemicals such as chlorine. The dominant method of electrochemical disinfection is to use oxidants produced during the electrochemical process – one of which is electro chlorination. Oxidant production in this method depends on several factors, such as electrode material, the chemical composition of electrolyte support, consumed electric current (or voltage), pH, temperature and type of electrolysis process [19–21]. The present study was conducted to determine the optimum conditions for removing bacteria and spores from drinking water and comparing them together.

2. Materials and methods

2.1. Microbial Strain

A strain of *Bacillus subtilis* (B. Subtilis ATCC 6633) grown on Trypticase soy broth (TSB) culture medium was prepared from the microbial collection available at the school. The spores were prepared by placing the strain in a temperature of about 36°C for 7 d. The microbial strain was transferred to Trypticase soy agar (TSA). The presence of spores was confirmed by typical colonies and malachite green staining [22] (Fig. 1).

The strains containing spores were stored and underwent electrochemical experiments at ambient temperature (about 25°C). The culture media and necessary materials were sterilized by autoclave for 15 min at 121°C. Prepared suspensions containing approximately 112 spore/mL were used. The pour plate method (CFU/mL) and transfer on TSA were used to evaluate the number of spores surviving after every 5 min of exposure to the electrochemical process. The plates were placed at a temperature of 25°C for 48 h.

The MPN/100 mL method was used to prepare a sample of water contaminated with *E. coli* (E. coli, ATCC32218) [23]. The lactose broth culture medium containing the bacteria was placed in a Bain–marie at 44.5°C for 24–48 h; then, the samples were transferred to EMB agar medium (Merck) and incubated at 35°C for 24 h. A contaminated water sample was prepared by adding a typical colony to 100 ml of water on a plate.

2.2. Electrochemical reactor

A 200-mL glass reactor was designed and prepared. A direct current power supply device was used to supply the required voltage. The electrodes were made of stainless steel at dimensions of 8 × 4 cm and spaced 2 cm apart from each other in the reactor (Fig. 2). Sampling was performed with a micro pipette at 5-min intervals to detect the surviving bacteria. The initial number of *E. coli*, *Bacillus subtilis* and *Bacillus subtilis* spores was 33, 112 and uncountable, respectively.

One cc of the prepared contaminated water sample was added to the reactor. In order to evaluate the effect of direct current on the disinfection process, the samples were exposed to different voltages (4.5, 6.5 and 8 V) and time intervals (5 and 30 min). The applied electric current

Fig. 1. Typical colonies of *Bacillus subtilis* spore grown on TSA culture medium.

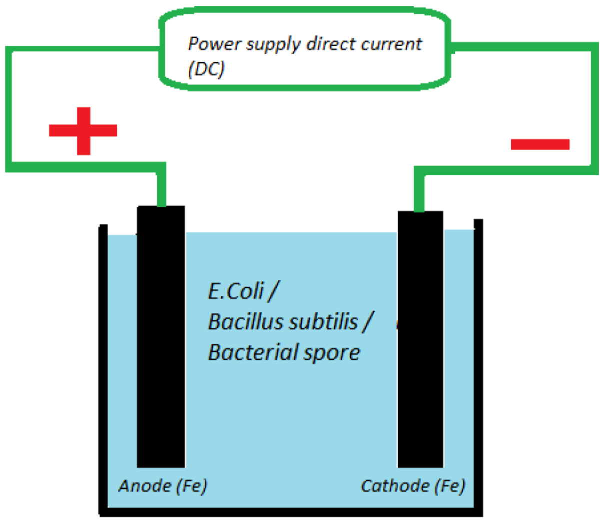

Fig. 2. The electrochemical reactor used in this research.

intensity (500 mA) was adjusted to the used voltage. The MPN/100 ml was determined at the beginning and end of the experiments. The pour plate method and transfer on TSA were used to count the number of *Bacillus subtilis* bacteria and spores surviving after the expiry of the electrolysis exposure time. The culture media were then placed in the incubator for 24 h, and the number of colonies was then counted using a colony counter. Table 1 presents the characteristics of the tested drinking water.

2.3. Findings

Table 2 presents the results of the experiment on *E. coli*, *Bacillus subtilis* and *Bacillus subtilis* spores. The efficiency of the electrolysis process in removing bacteria increases with applied voltage and electrochemical contact time. Figs. 1 and 2 show the effect of different voltages on the growth of *Bacillus subtilis* and *E. coli* bacteria, respectively. Figs. 3 and 4 show a comparison of the survival rates of *E. coli* and *Bacillus subtilis* at 4.5 and 8 V, respectively. Electric current had a positive effect on the growth of *E. coli*, *Bacillus subtilis* and *Bacillus subtilis* spores. In this study, the number of colonies of both bacteria decreased over time. The results of the experiments showed that the resistance of *E. coli* is much lower than that of *Bacillus subtilis* bacteria and *Bacillus subtilis* spores. The results of examining the effect of electric current on bacterial and spore elimination showed that the number of surviving colonies has an inverse correlation with time.

Table 2 indicates that the efficiency of the electrochemical process is better in the removal of *E. coli* and requires more time for *Bacillus subtilis* removal. Examining the impact of the time and voltage of the exposure of the bacteria showed that increased time and increased voltage have a significant effect on the reduction of the bacteria. The effect of electric current was greater on *E. coli* bacteria than *Bacillus subtilis* bacteria and spores.

Table 1
Water characteristics

Parameter	Amount
Magnesium, mg/L	20.4
Calcium, mg/L	57.8
Sodium, mg/L	91
Potassium, mg/L	1.3
Ammonia nitrogen, mg/L	0.1
Electrical conductivity(µs/cm)	638
Chloride, mg/L	36.1
Sulfate, mg/L	22
Fluoride, mg/L	0.26
Nitrate, mg/L	12
Total soluble solids, mg/L	631
pH	7.4

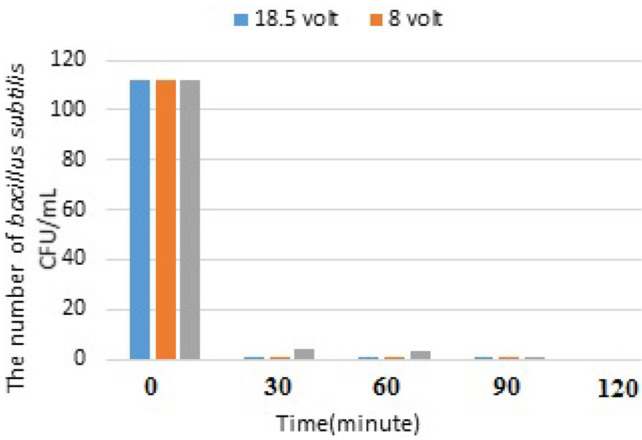

Fig. 3. The number of surviving *Bacillus subtilis* (CFU/mL) in an electrical current of 500 mA.

Table 2
The effects of electricity on the growth of *E. coli*, *Bacillus subtilis* and *Bacillus subtilis* spores at 4.5, 6.5 and 8 V with an electric current of 500 mA

Voltage	Bacterial species	Contaminated water before electrochemical disinfection	30 min after electrochemical disinfection	60 min after electrochemical disinfection	90 min after electrochemical disinfection	120 min after electrochemical disinfection
4.5 V	*Escherichia Coli*	33	0	0	0	0
	Bacillus subtilis	112	4	3	1	0
	Spore Bacillus subtilis	Uncountable	52	38	19	2
6.5 V	*Escherichia Coli*	33	0	0	0	0
	Bacillus subtilis	112	3	2	0	0
	Spore Bacillus subtilis	Uncountable	49	31	13	0
8 V	*Escherichia Coli*	33	0	0	0	0
	Bacillus subtilis	112	1	1	1	0
	Spore Bacillus subtilis	Uncountable	43	25	8	0

The results of examining the effect of electrolysis time on the removal efficiency of *E. coli* and *Bacillus subtilis* bacteria and spores showed that removal efficiency has a direct correlation with time.

3. Discussion

Oxidation and reduction in the electrochemical process are carried out in the anode and cathode electrodes, respectively, conjoint with the production of hydrogen peroxide oxidants in the cathode, ozone in the presence of oxygen and free chlorine and chlorine dioxide in the presence of chloride in the anode. The main products in the anode are oxygen and chlorine, which are associated with the acidification of water near the anode. The main products in the cathode are calcium carbonate and magnesium hydroxide deposits [2]. The electrochemical process is quite complex in liquid systems. According to relations 1, 2 and 3 on the anode and cathode electrodes, oxidation and reduction are performed as follows [21]:

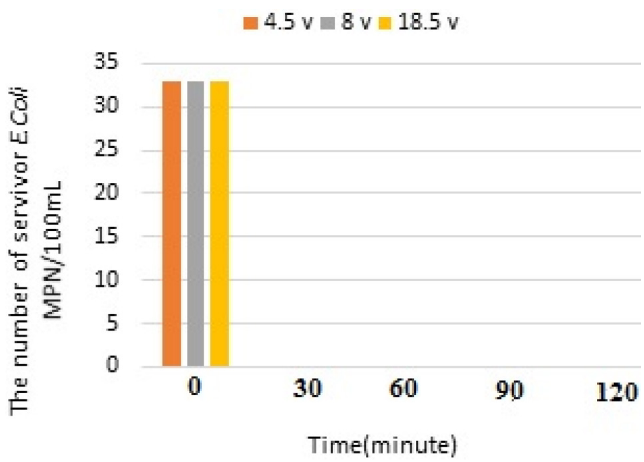

Fig. 4. The number of surviving *E. coli* (MPN/100 mL) in an electrical current of 500 mA.

$$\text{Cathode: } H_2O + e^- \rightarrow + OH^- + \frac{1}{2}H_2 \quad (1)$$

$$\text{Anode: } Cl^- \rightarrow ½\, Cl_2 + e^- \quad (2)$$

$$H_2O + Cl^- \rightarrow OH^- + ½\, H_2 + ½\, Cl \quad (3)$$

Electrochemical technology is a processing unit in which the mineralization of bacteria occurs through electron transfer reactions at the interface of electrode and water [24].

The results of the experiment showed that the number of *E. coli*, *Bacillus subtilis* and *Bacillus subtilis* spores increases with time and voltage. The bacterial resistances are:

E. coli < *Bacillus subtilis* < *Bacillus subtilis* spores

It is evident that the bactericidal effect of the process depends on the time of electrochemical contact and the applied voltage of the system. As shown, *Bacillus subtilis* bacteria are completely eliminated after 120 min at different voltages. In a study entitled 'Chicken slaughterhouse wastewater disinfection by electrocoagulation using copper electrode', Zarei et al. found that the electrocoagulation process using copper electrodes was able to fully remove the total coliform from the chicken slaughterhouse wastewater [25]. In a study to remove humic acid by electrocoagulation from natural aqueous environments, Rezaei et al. found that an electrochemical process with iron electrodes can effectively remove humic acid from the aqueous medium [26]. In a study on the removal of humic acid from water environments by electro coagulation using iron electrodes, Bazrafshan et al. found that electro coagulation with Fe electrodes can successfully remove HA from aqueous environments. Also, increasing the contact time up to 15–75 min increased the process efficiency [27]. Rahmani et al. investigated bacterial elimination by electrolysis and found that the amount of removal would depend on the voltage and duration of electrolysis and that the removal efficiency is higher at higher

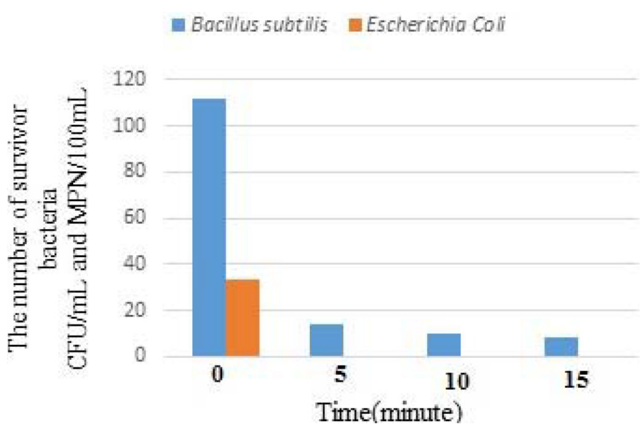

Fig. 5. The number of surviving bacteria (*E. coli* and *Bacillus subtilis*) in an electrical current of 500 mA at a voltage of 4.5 V.

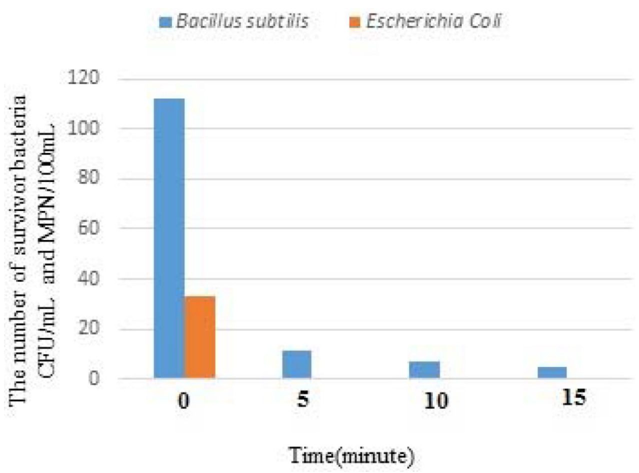

Fig. 6. The number of surviving bacteria (*E. coli* and *Bacillus subtilis*) in an electrical current of 500 mA at a voltage of 8 V.

voltages [28]. The bacterial mortality during the electrochemical process involves the production of chemical disinfectants such as chlorine and ozone, flux transfer in the bacteria-electrode interface, the destruction of the cytoplasmic membrane and increased cell membrane permeability [29]. In a study by Rahmani and Samarghandi on the efficacy of removing coliform bacteria from drinking water by electrolysis, removal efficiency was found to depend on the voltage and material of the electrodes. According to the results of the experiments, the best disinfection efficiency pertains to stainless steel electrode pairs placed 2 cm apart from each other at a voltage of 10 volts and a current of 135 mA, in which MPN/100 mL = 0 was obtained over 5 min [10]. Increasing the voltage and electrolysis time led to increased disinfection efficiency due to the faster production of electrolysis products such as CL^- and OH ions in the cathode and anode electrodes, respectively. The intended products are responsible for the disinfection of water [30]. These studies are consistent with the study by Rahmani in 2005 [28]. Rahmani showed that increasing the voltage from 8 to 25 V and reducing the distance from 8 to 2 cm leads to an increase in the efficiency of *E. coli* bacteria removal at the rate of 1 colony/liter [10]. In a study conducted by Petrovsky et al. in 2005, a significant reduction was reported in the number of bacteria after examining the effect of electrical stimulation on the growth of *E. coli* at a voltage of 20 mA over 30 min, which is consistent with the results of the present research [20].

4. Conclusion

The results of this study showed that the sensitivity of different microorganisms to electric current and voltage varies. The electrochemical disinfection method eliminates the hazards of conventional water disinfection methods. The reactor used in this research was able to eliminate *Bacillus subtilis* spores after the expiry of 2 h.

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References

[1] S. Hoseini, K.S. Amiri, K.S. Hashemi, Effects of electrical current on fungal and bacterial removal from water, 2016.
[2] A. Rezaee, G. Kashi, A.J. Jafari, A. Khataee, Investigation of *E. coli* removal from polluted water using electrolysis method, Iran. J. Health Environ., 4 (2011) 201–212.
[3] V. Alipour, L. Rezaei, M.R. Etesamirad, S. Rahdar, M.R. Narooie, A. Salimi, J. Hasani, R. Khaksefidi, S.A. Sadat, H. Biglari, Feasibility and applicability of solar disinfection (SODIS) for point-of-use water treatment in Bandar Abbas, South of Iran, J. Global Pharm. Technol., 9 (2007) 40–46.
[4] M. Malakotian, S.Y. Hashemi, New methods analysis of nitrate removal from water resources, J. Toloo–e–behdasht, (2015/3).
[5] M. Afsharnia, H. Biglari, S.S. Rasouli, A. Karimi, M. Kianmehr, Sono-electro coagulation of fresh leachate from municipal solid waste; simultaneous applying of iron and copper electrodes, Int. J. Electrochem. Sci., 13 (2018) 472–484.
[6] R. Rostami, K. Naddafi, A. Aghamohamadi, Survey of peanut fungal contamination and its relationship with ambient conditions in the Bazar of Zanjan, 2009.
[7] M. RadFard, H. Biglari, H. Soleimani, H. Akbari, H. Akbari, H. Faraji, O. Dehghan, A. Abbasnia, M. Hosseini, A. Adibzadeh, Microbiological dataset of rural drinking water supplies in Zahedan, Iran, Data in Brief, 20 (2018) 609–613.
[8] M. Yousefi, H.N. Saleh, M. Yaseri, A.H. Mahvi, H. Soleimani, Z. Saeedi, S. Zohdi, A.A. Mohammadi, Data on microbiological quality assessment of rural drinking water supplies in Poldasht county, Data in Brief, 17 (2018) 763–769.
[9] S.M.R.R. Alireza, Investigation of coliform removal from drinking water by electrolysis, Scient. J. Hamadan Univ. Med. Sci. Health Services, 15 (summer 2008).
[10] M. Yousefi, H.N. Saleh, M. Yaseri, M. Jalilzadeh, A.A. Mohammadi, Association of consumption of excess hard water, body mass index and waist circumference with risk of hypertension in individuals living in hard and soft water areas, Environ. Geochem. Health, (2018) 1–9.
[11] M. Fazlzadeh, H. Sadeghi, P. Bagheri, Y. Poureshg, R. Rostami, Microbial quality and physical–chemical characteristics of thermal springs, Environ. Geochem. Health, 38 (2016) 413–422.
[12] A. Takdastan, M. Mirzabeygi, M. Yousefi, A. Abbasnia, R. Khodadadia, H. Soleimani, A.H. Mahvi, D.J. Naghan, Neuro-fuzzy inference system prediction of stability indices and sodium absorption ratio in Lordegan rural drinking water resources in west Iran, Data in Brief, 18 (2018) 255–261.
[13] P. Setlow, Germination of spores of Bacillus species: what we know and do not know, J. Bacteriol., 196 (2014) 1297–1305.
[14] A.M. Arjmand, M. Rezaee, S. Naseri, S. Eshraghi, Study of sodium chloride supporting electrolyte on electrochemical removal of *Bacillus subtilis* spores from drinking water, Iran. J. Health Environ., 8 (2015) 81–88.
[15] M. Afsharnia, M. Kianmehr, H. Biglari, A. Dargahi, A. Karimi, Disinfection of dairy wastewater effluent through solar photo catalysis processes, Water Sci. Eng., 11 (2018) 214–219.
[16] M. Yousefi, M. Yaseri, R. Nabizadeh, E. Hooshmand, M. Jalilzadeh, A.H. Mahvi, A.A. Mohammadi, Association of hypertension, body mass index, and waist circumference with fluoride intake; water drinking in residents of fluoride endemic areas, Iran, Biological trace element research, (2018) 1–7.
[17] R. Khosravi, M. Fazlzadehdavil, B. Barikbin, H. Hossini, Electro–decolorization of Reactive Red 198 from aqueous solutions using aluminum electrodes systems: modeling and optimization of operating parameters, Desal. Water Treat., 54 (2015) 3152–3160.
[18] R. Khosravi, H. Hossini, M. Heidari, M. Fazlzadeh, H. Biglari, A. Taghizadeh, B. Barikbin, Electrochemical decolorization of reactive dye from synthetic wastewater by mono-polar aluminum electrodes system, Int. J. Electrochem. Sci., 12 (2017) 4745–4755.
[19] R.A.A. Hasanbiki, H. Masoumbigi, H. Hossini, Removal of *Escherichia coli* from contaminated water using bipolar electrochemical systems, J. North Khorasan Univ., (2015) 551–560.
[20] R. Khosravi, S. Hazrati, M. Fazlzadeh, Decolorization of AR18 dye solution by electro coagulation: sludge production and electrode loss in different current densities, Desal. Water Treat., 57 (2016) 14656–14664.
[21] S. Gholami, Investigation of the survival of bacteria under the influence of supporting electrolytes KCl, CuI and NaBr in the electrochemical method, Mental Health, 4 (2018) 104–111.
[22] M. Cho, J.-H. Kim, J. Yoon, Investigating synergism during sequential inactivation of *Bacillus subtilis* spores with several disinfectants, Water Res., 40 (2006) 2911–2920.
[23] E. Rice, R. Baird, A.L.S. Eaton, Standard methods: For the examination water and wastewater, 22^{nd} edn. American Public Health Association, American Water Works Association, Water Environmental Federation., 2012.
[24] G. Kashi, N. Hejazimehr, S. Yavarpour, Investigation of pseudomonas aeruginosa removal from drinking water using photo-electrochemical method, J. Health Field, 1 (2017).

[25] A. Zarei, H. Biglari, M. Mobini, A. Dargahi, G. Ebrahimzadeh, M.R. Narooie, E.A. Mehrizi, A.R. Yari, M.J. Mohammadi, M.M. Baneshi, Disinfecting poultry slaughterhouse wastewater using copper electrodes in the electro coagulation process, Polish J. Environ. Stud., 27 (2018) 1907–1912.
[26] H. Rezaei, M.R. Narooie, R. Khosravi, M.J. Mohammadi, H. Sharafi, H. Biglari, Humic acid removal by electro coagulation process from natural aqueous environments, Int. J. Electrochem. Sci., 13 (2018) 2379–2389.
[27] E. Bazrafshan, H. Biglari, A.H. Mahvi, Humic acid removal from aqueous environments by electro coagulation process using iron electrodes, J. Chem., 9 (2012) 2453–2461.
[28] R. Tolentino-Bisneto, E.D. Bidoia, Effects of the electrolytic treatment on *Bacillus subtilis*, Brazil. J. Microbiol., 34 (2003) 48–50.
[29] A. Kraft, Electrochemical water disinfection: a short review, Platinum Metals Rev., 52 (2008) 177–185.