Evaluation of treatment and disinfection of water using cold atmospheric plasma

Zohreh Rashmei, Hamid Bornasi and Mahmood Ghoranneviss

ABSTRACT

In this paper, the disinfection of water is investigated using plasma spark treatment and the results are compared with conventional techniques. Inactivation of the *Enterococcus faecalis* and *Escherichia coli* bacteria is considered in the treatment process of water by the plasma spark. For this purpose, many physical and chemical parameters of water are measured and the obtained results demonstrate a reduction of 8-log in colony forming units of *E. coli* and *E. faecalis* at 15 minutes and 12 minutes, respectively. The results of this research show that no ozone is produced during the plasma spark treatment. Moreover, inactivation of a large number of bacteria without any change of pH shows that pH is not the cause of the bacterial inactivation. It is concluded that the main causes of the inactivation of bacteria in the treated water are H_2O_2 molecules and the electrical fields generated by plasma.

Key words | cold atmospheric plasma, disinfection, *Enterococcus faecalis*, *Escherichia coli*, plasma, water

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INTRODUCTION

The quality of water has a substantial role in public health. Contaminated water can cause disease outbreaks and serve as a mechanism to transmit communicable diseases. The World Health Organization (WHO) estimated that diarrhoeal disease claimed the lives of 2.5 million people annually (WHO 2013). The conventional method of water disinfection is using chlorine. In recent years, due to the side effects and the environmental hazards of chlorine, the use of ozone has replaced it and become more common (Glaze *et al.* 1987). However, due to the absence of residual ozone in water, it is unable to eliminate secondary pollution (Liu *et al.* 2010; Grellier *et al.* 2015).

The use of plasma is one of the new techniques that has recently attracted attention among researchers into water disinfection (Fridman *et al.* 2007; Oehmigen *et al.* 2010). Since in this method no additional substance is employed in the water, it is highly interesting to scientists (Mededovic & Locke 2007; Kim *et al.* 2014). Plasma can be produced by a variety of electrical discharges. All plasma systems, in terms of electronic density or temperature, are divided into two major categories, namely thermal and non-thermal (Joshi *et al.* 1995; Babaeva *et al.* 2014; Jiang *et al.* 2014).

One of the new applications of plasma is the inactivation of microorganisms (Gibson *et al.* 2011; Akan & Çabuk 2014). The inactivation effect has been attributed to the presence of active plasma species (OH, O, O_3, H_2O_2, UV, and NO), decreased pH and electric fields generated by the discharge of plasma (Julák *et al.* 2012; Johnson *et al.* 2015). Each of these factors plays a significant role in the inactivation of microorganisms (Guo *et al.* 2015). Various types of plasma discharges, such as dielectric barrier discharge, corona discharge and spark discharge plasma, were reported to be able to produce active plasma species (Li *et al.* 2014; Kim *et al.* 2015).

Recently, Kim *et al.* (2013) used gliding arc discharge for inactivation of *Escherichia coli* in water. They observed 5-log reductions in the colony forming unit (CFU) number of *E. coli* in 20 min. They also stated that the main cause

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of microorganism inactivation is the creation of hydrogen peroxide molecules. The inactivation of bacteria in water using atmospheric plasma was studied by Liu *et al.* (2010). They achieved a 100% inactivation rate of *Staphylococcus aureus* in 16 min. In their study, the decrease in pH with the increase in oxidation of perhydroxyl radicals ($HOO^{\cdot}$) was introduced as the main cause of bacterial death.

In the present study, there are three main objectives. First, the inactivation of two main bacteria in water pollution (i.e., *E. coli, Enterococcus faecalis*) using spark plasma is under investigation. Inactivation of *E. faecalis* in water by plasma is studied for the first time in the world. The bacteria *E. coli* and *E. faecalis* are important indicators of water pollution. At the second step, the principal cause of water disinfection treated by plasma is studied. The final aim is to assess the side effects of plasma in water, to which less attention has been devoted in the literature until now.

METHODS

In this section, the three main objectives mentioned above are studied in the following subsections. In the first section, water disinfection with a short description of the experimental procedure is presented, and in the two following sections the detection of the cause of disinfection and its side effects are explained.

Disinfection of water

The spark discharge plasma used for the disinfection of water consists of two separated needle electrodes and a pulsed power supply (10 kV @ 30 Hz). The non-thermal plasma (NTP) is generated by a simple apparatus of an open-air type. The two needle electrodes are separated from each other by 5 cm, and the distance of the electrodes from the water surface is 4 mm.

Our experiments were conducted by plasma injection. In this system, the bacteria are suspended in the water poured in a shallow container (whose volume is typically 10 mL) and the NTP is applied to the surface of the liquid. Two different bacterial cell wall structures were used in the present study. Gram-positive bacteria *E. faecalis* and Gram-negative *E. coli*, isolated from water and by microbiological and immunological methods, were identified and were used as the test organism.

E. coli and *E. faecalis* were cultured by PCA (plate count agar). Fresh cultures of bacteria colonies were used for making the bacterial suspension in water and PBS (phosphate buffered saline, 2.7 mM KCL, 137 mM NaCl, 1.5 Mm KH_2PO_4 and 8.1 mM Na_2PO_4). In order to make the initial concentration of the bacteria suspension, 0.5 McFarland standard was used as well. The initial cell concentration was controlled at 10^8 CFU/mL. The initial cell concentration and cell viability were measured by culture and by a dilution of 10^{-6}. The CFU count of bacteria with different dilutions after plasma treatment for various time durations in a range of 1, 3, 5, 7, 10, 12 and 15 min was evaluated. The PCA samples were incubated for 24 hr at 35 °C and then the number of colonies was counted.

Detection of disinfection factor

The main possible causes of disinfection in the water treated by plasma (increasing temperature, decreasing pH, hydrogen peroxide, ozone, and nitric oxide) were investigated. The physical and chemical parameters were measured in untreated water ($t = 0$ min) and the plasma-treated water ($t = 1, 3, 5, 7, 10, 12, 15$ min).

pH

pH was measured using a Metrohm pH-meter (Model 744) apparatus with a glass microelectrode.

H_2O_2

The concentration of H_2O_2 in water was detected based on the colorimetric method. It was measured using a peroxide test strip (EMD Chemicals, Germany) and by observing the colour change in the strip.

Temperature

The changes in temperature were measured using a digital thermometer (Testo- T1).

O_3

The concentration of O_3 in the total samples was evaluated using the CHECKIT Comparator kit, based on the colorimetric measurement of colour product of the ozone reaction with *N*,*N*-diethyl-1,4-phenylenediamine (DPD)/potassium iodide.

Plasma side effects

The side effects of plasma in water were considered in previous studies by the investigation of nitrate and nitrite anions alone, while in the present paper many more substantial parameters of the quality of drinking water were considered.

$NO^{-3}/NO^{-2}/SO_4^{-2}$

The concentrations of nitrate anions (NO^{-3}), nitrite anions (NO^{-2}) and sulfate anions (SO_4^{-2}) in untreated and plasma-treated water were measured based on the spectroscopic method and using a spectrophotometer (HACH, DR-5000) and nitrate (Cat: 21061-69), nitrite (Cat: 21071-69) and sulfate (Cat: 21067-69) kits.

Dissolved oxygen

Dissolved oxygen (DO) was determined using the DO-Meter (JENCONS, 970) apparatus with the a membrane electrode.

Turbidity measurements

Turbidity in the water was evaluated by a turbidity meter (WTW, TURB 355 IR).

Total hardness

Total hardness ($CaCo_3$) was detected based on the reaction of ethylene diamine tetraacetic acid.

Conductivity and total dissolved solids

Water conductivity and total dissolved solids (TDS) were measured by a conductivity meter (Metrohm – 744) with a measuring range of 1 μS/cm to 100 mS/cm.

RESULTS

Disinfection of the water

The bactericidal effect of the water exposed to spark discharge for 1, 3, 5, 7, 10, 12 and 15 min and then stored for 24 hr in a refrigerator is shown in Figures 1 and 2.

In the case of water (i.e., with an initial concentration of 10^8 CFU/mL), there is an 8-log reduction in the CFU count of *E. coli* during the 15 min plasma treatment and there is an

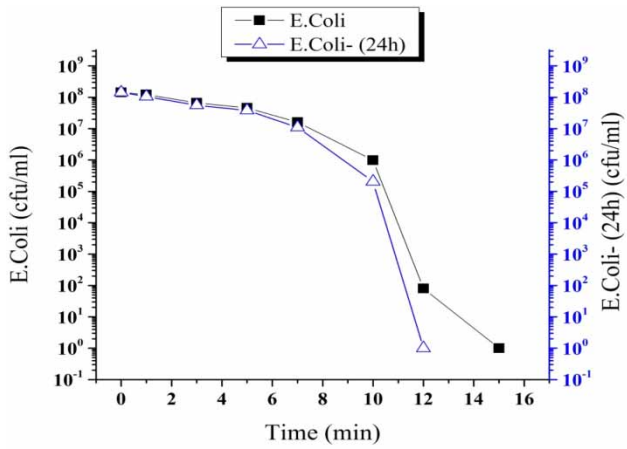

Figure 1 | Results of *E. coli* inactivation in water exposed to spark discharge and stored for 24 hr in a refrigerator.

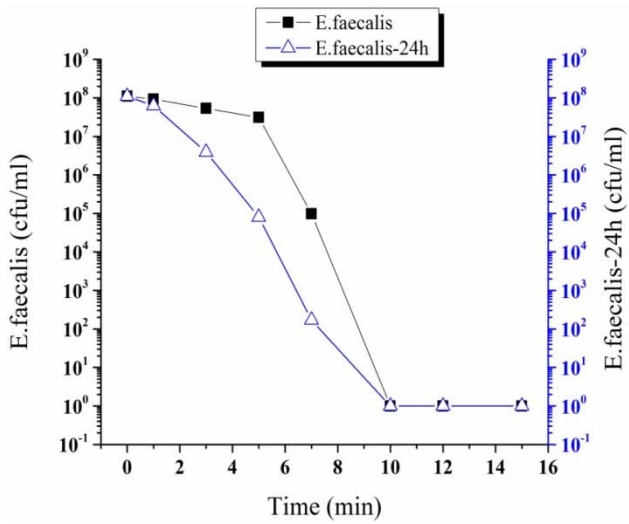

Figure 2 | Results of *E. faecalis* inactivation in water exposed to spark discharge and stored for 24 hr in a refrigerator.

additional 1-log reduction during the storage period of 12 min in Figure 1. After a 12 min treatment of water, there is an 8-log reduction in the CFU count of *E. faecalis*. In addition, a 3-log reduction during the storage period (24 hr) of 10 min was achieved (Figure 2).

The complete inactivation of *E. faecalis* was observed after 12 min of incubation in 10 mL of exposed water. In contrast, the complete inactivation of *E. coli* needed 15 min of incubation. In the tests with plasma treatment, the *E. coli* and *E. faecalis* concentrations consistently dropped during the period of treatment in water. With the same initial concentration as water, there is an 8-log reduction in the CFU count during plasma treatment of 12 min in the PBS case and also there is an additional 8-log reduction of the CFU count during 3 min of plasma treatment with a storage period of 24 hr. It can be concluded that the PBS significantly improved the bacterial inactivation during the storage period (Figure 3).

Similar graphs are also plotted for other experimental conditions (stored and fresh water and PBS, exposed to spark discharge). PBS exposed to spark discharge exhibits complete inactivation in the 10 mL of exposed water. A similar difference between buffered and water cases can be found in regard to the inactivation of microorganisms by the spark plasma action. *E. coli* in water is completely inactivated after 15 min of incubation in 10 mL of exposed water.

Investigation of the disinfection factor

Temperature

Changes in water temperature before and after the plasma radiation and PBS show that the temperature increases to 37 °C and 45 °C.

pH measurements

A sudden drop in pH is observed in the water at short time exposure. The pH of the water decreases from an initial mean value of 8.43 ± 0.10 to 4.15 ± 0.1 at the end of plasma treatment. In addition, a significant change of pH is observed as well in all the exposed samples after 24 hr storage (Figure 4). No major change of pH was observed in PBS. The pH under plasma spark remains almost unchanged, with the minimal pH 7.14 in 10 mL (Figure 5).

Hydrogen peroxide determination

The concentration of H_2O_2 in water was measured over 15 min so as to determine the effective residual time. The results of H_2O_2 concentrations in water in the form of colour changes in peroxide test strips show the increasing

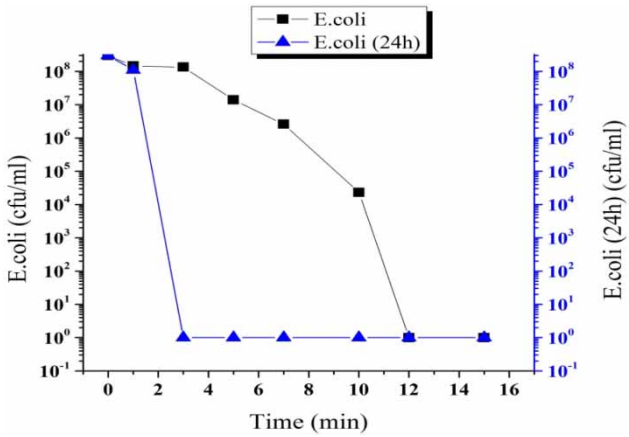

Figure 3 | Results of *E. coli* inactivation in PBS exposed to spark discharge and stored for 24 hr in a refrigerator.

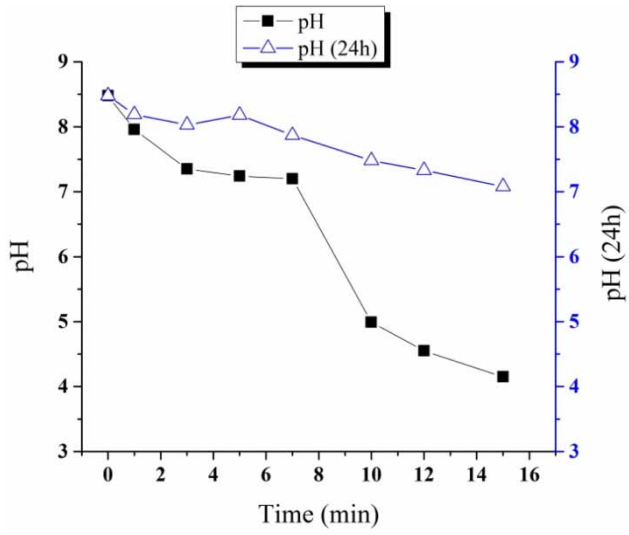

Figure 4 | Decrease in pH, when plasma was exposed to water and change of pH after storage for 24 hr.

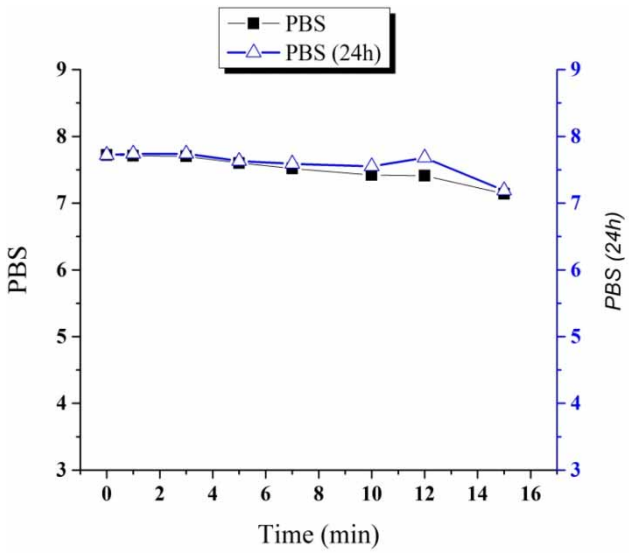

Figure 5 | Buffer pH remained unchanged.

of H_2O_2 content in all samples with the growth of exposure time up to 100 mg/L under the plasma spark in 10 mL of water. In all samples, for exposed plasma sparks stored for 24 hr in a refrigerator, the H_2O_2 content did not decrease. The maximum H_2O_2 concentration with the peroxide test strips is 100 mg/L, as shown in Figure 6.

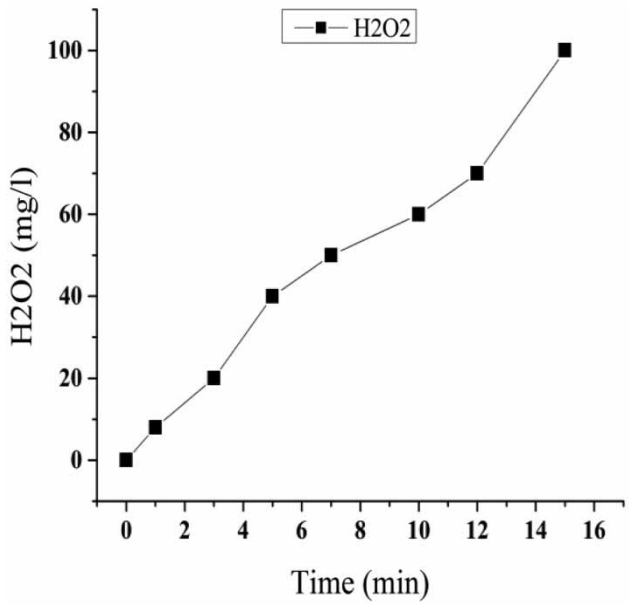

Figure 6 | Increase in H_2O_2 concentration, when plasma was exposed to water.

O_3

After plasma irradiation, the initial concentration (0 mg/L) of ozone remained unchanged, therefore plasma does not have the capability of producing ozone.

Plasma side effects

The side effects of plasma in water were also studied. The results show that the plasma has little effect on parameters including total hardness, turbidity, sulfate, TDS, conductivity and DO. As a result of plasma radiation there was an increase of nitrate and nitrite concentrations in water (Figure 7).

DISCUSSION

Contaminated water is the main cause of many infectious diseases. According to the latest report from the World Health Organization, each year about 2.5 million people die due to bloody diarrhea caused by contaminated water (WHO 2013). Disinfection of microorganisms is an important step in the water treatment processes (Shaw *et al.* 2014). Disinfection by chlorination is a common technique (Moore 2014). The main concern in chlorine treatment methods is THM (trihalomethane) compounds, due to the increase in the risk of cancer (Dore 2015; Teo *et al.* 2015).

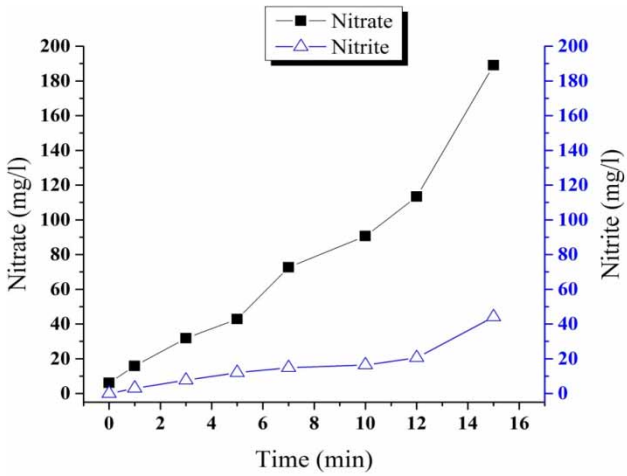

Figure 7 | Increased nitrate and nitrite concentrations in water exposed to plasma.

Ozone is a remarkably versatile and powerful disinfectant, although the need to produce it on site makes it expensive and there is a lack of residual disinfection capability in comparison to alternatives such as chlorination or UV (Rajab *et al.* 2015). While previous researchers have explored the effect on bacterial inactivation by active species plasma (Sugiarto *et al.* 2002), this study is mainly focused on the inactivation of microorganisms, the investigation of disinfection factors and the side effects in plasma-treated water with spark discharge.

In particular, the study investigates the efficiency of plasma in the inactivation of indicator bacteria in water pollution (*E. coli, E. faecalis*). The results show that plasma has the capability to destroy high concentrations of *E. coli* and *E. faecalis* in water. *E. faecalis* compared with *E. coli* bacteria is inactivated in less time. This can be due to the different structure of the cell wall of the two bacteria (Figure 8).

The cell walls of Gram-negative bacteria (*E. coli*) are much more complicated than the walls of Gram-positive bacteria (*E. faecalis*). Gram negatives contain the major components of lipopolysaccharide, lipoprotein, and relatively little peptidoglycan (<10% of the total cell wall) in their cell walls, whereas the walls of Gram positives are mainly composed of peptidoglycan (usually 30–70% of the total cell wall), polysaccharides, teichoic acid or teichuronic acid (Schleifer & Kandler 1972). As mentioned, the polymers making up the cell walls are chemically extremely different in these two groups of bacteria, which can have an effect on the entry capability and the influence of plasma active species. The inactivation effect of bacteria was observed for the exposed water stored for 24 hr as well. In addition, 1 and 3-log reduction was observed during the storage period for *E. coli* and *E. faecalis*, respectively, due to the stability of the active plasma species.

In this study, the production of disinfection in water treated by plasma is measured. The results of measurement during plasma radiation show that the small temperature rise, up to 45 °C, cannot be the cause of the inactivation of microorganisms. In previous studies, the pH of water during exposure to plasma decreased due to the presence of H^+ (Chen *et al.* 2009; Julák *et al.* 2012). These findings roughly correspond with our results. Also, in our study, the value of pH in PBS exposure to plasma was measured. The pH of the buffer is not reduced while the inactivation of bacteria in the buffer is greater than in water. Although hydrogen peroxide in acidic conditions is a strong oxidizer and can have a greater impact on the bacterial cell membrane, the present results demonstrate that it cannot be attributed to the acidification alone.

The inactivation rate (Figures 9 and 10) is a non-dimensional parameter, and is the difference of the concentrations (CFU/mL) of bacteria before and after plasma radiation in each duration time to the initial concentrations of the bacteria.

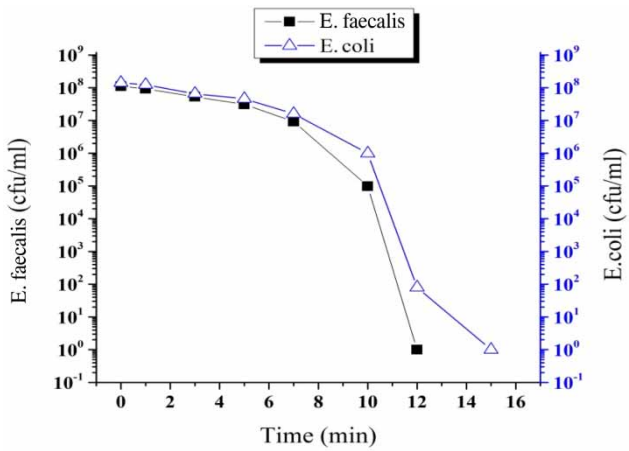

Figure 8 | Comparison of inactivation of *E. coli* with *E. faecalis* in water exposed by plasma.

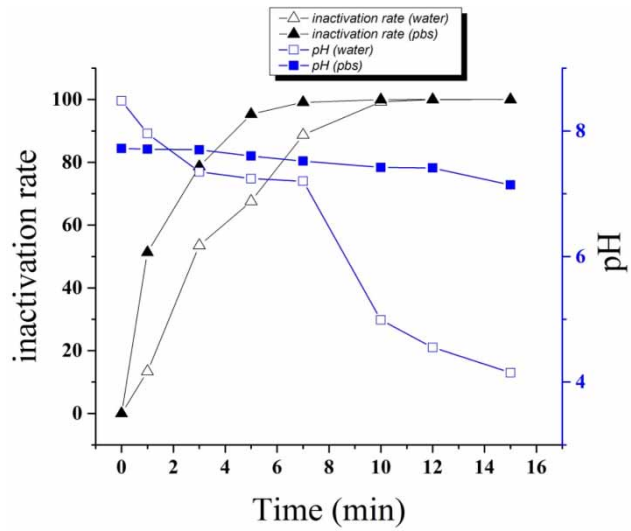

Figure 9 | Inactivation of *E. coli* as compared with variations of pH in exposed water and PBS.

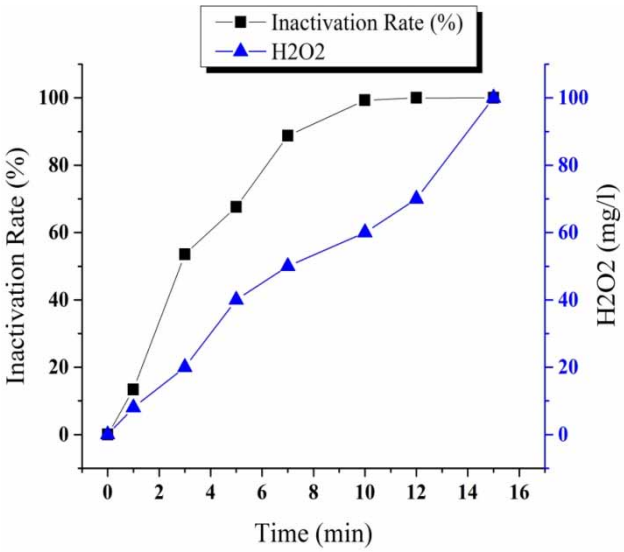

Figure 10 | Inactivation of *E. coli* as compared with increased H_2O_2 concentrations in exposed water.

It is calculated by the following formula:

$$\frac{N2 - N1}{N1} \times 100$$

where $N1$ is the initial concentration (CFU/mL) of the bacteria before plasma radiation and $N2$ is the concentration of bacteria (CFU/mL) after plasma radiation.

Ozone, as one of the active plasma species, is not generated only due to the plasma radiation on the water surface. Based on the results of this paper, the concentration of nitrate and nitrite increased steadily with the exposure time in water. These results were consistent with previous studies (Liu *et al.* 2010). Investigation of other parameters did not show any significant changes in the water exposed to plasma.

Experimental results obtained in this study indicate that the spark discharge reacting with water can effectively generate H_2O_2. When the water is directly exposed to a spark discharge, the reactions can occur with the dissociation of water molecules, and then H_2O_2 can be produced from the recombination of hydroxyl radicals (Kim *et al.* 2014). Therefore, the formation of H_2O_2 molecules confirms the existence of hydroxide radicals in the water. Hydrogen peroxide (H_2O_2) demonstrates broad-spectrum efficacy against viruses, bacteria, yeasts and bacterial spores. Thus it is widely used as a biocide, particularly in medical, food and industrial applications (Linley *et al.* 2012). Hydroxide radicals and H_2O_2 with the electrical potentials of 2.8V and 1.78V are powerful oxidizers which attack essential cell components, including lipids, proteins and DNA. In general, greater activity is seen against Gram-positive than Gram-negative bacteria (McDonnell & Russell 1999; Block 2001). Hence it can be said that *E. faecalis* is destroyed faster than *E. coli* due to the presence of hydrogen peroxide in the water exposed to plasma.

CONCLUSIONS

In the present paper, the disinfection of water using plasma spark treatment is investigated. The inactivation of bacterial indicators in water pollution, the major cause of inactivation of bacteria and its side effects are also studied. The results show that the plasma has a strong capability to kill bacteria. 8-log CFU reductions in the concentration of both species are observed, and a few seconds of radiation lead to the death of millions of bacteria. It is demonstrated that the plasma destroying ability for Gram-positive bacteria is greater than for Gram-negative bacteria.

The main factors in plasma bactericidal activity are due to the consequence of electric field generation and the appearance of H_2O_2 molecules created by the combination of hydroxyl radicals. H_2O_2 molecules and hydroxyl radicals are very strong oxidizers that have the ability to inactivate bacteria efficiently. It is also observed that pH reduction and other factors have no effect on the inactivation of bacteria. The study of plasma side effects shows that the physical and chemical parameters (total hardness, turbidity, sulfate, TDS, conductivity and DO) change a little. An increase in the concentration of nitrate and nitrite in water treated by plasma is an important side effect of this method. In order to prevent the generation of nitrate and nitrite, it is suggested to provide a new experimental procedure taking into account considerations such as vacuum conditions, lack of nitrogen gas or the use of different electrodes.

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