# Point-to-plane liquid phase plasma discharge with copper electrode for *Escherichia coli* inactivation in water

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**Abstract:** The application of point-to-plane direct-in-liquid arc plasma discharge with copper electrodes in treating a synthetic water sample spiked with *Escherichia coli* (ATCC 25922) was investigated. Two control experiments, with no plasma exposure, for every plasma run were conducted, one with ionic copper and the second with no copper. For initial $1.3 \times 10^5$ *Escherichia coli* colony-forming units per millilitre of water, a 0.8 log reduction for ionic copper after 300 seconds and a 3.4 for plasma after 60 seconds was observed. In a second experiment with initial $3.9 \times 10^4$ *Escherichia coli* colony-forming units per millilitre of water, a 1.7 log reduction for ionic copper after 300 seconds and a 4.5 for plasma after 60 seconds was observed.

**Keywords:** Escherichia coli, inactivation, plasma, copper

## 1. Introduction

More than 1.1 million global deaths each year are attributed to the consumption of water-borne pathogen contaminated water and pose a health risk, especially to people with depressed immunity [1].Chlorination and ultraviolet (UV) radiation are common disinfection methods with potential harmful by-products and high energy consumption respectively. Advanced oxidation processes are water-borne pathogen inactivation technologies with promise though they are still in research and developmental stages [2].

The inactivation of *Escherichia coli* by liquid-phase direct plasma has been demonstrated [3]. For this study, *Escherichia coli* was chosen as it has become a standard pathogen for inactivation studies and there are emerging strains which are a cause of concern [4]. Liquid-phase direct plasma effectiveness is a function of electrode material, electrode polarity, voltage and current settings of the power supply. This study is a follow up on previous investigations where the electrode polarity, current and voltage settings were determined. Previous experiments used a tungsten cathode but electrode wear resulted in the final water having a milky white colour. The choice of the copper electrode in this study was influenced by the fact that copper is an excellent electric conductor, has antimicrobial properties and dissipates heat quickly, hence copper stays cooler and reduce electrode wear [5]. Most studies do not provide information on the potential contribution of the electrode material used in the generation of the electrical discharge, but often, inactivation appears to be solely the effect of plasma without investigating the influence of the electrode interaction with the microbe. This study further investigates the contribution of released copper ions into the water during treatment by having two control reactors not exposed to plasma.

## 2. Materials and Method

### 2.1 Experimental setup

The plasma experiments were conducted in a 500 ml capacity quartz reactor with an operating water volume of 300 ml. The electrodes were made of solid copper (d=6 mm) with the top electrode having a $60^0$ angle tip and being the high voltage electrode (cathode) in negative polarity. The electrode gap was 4 mm. An arc discharge was generated between the two copper electrodes at atmospheric pressure by applying a high voltage to the cathode using a Technix-SR-10R-5000 supplying direct current (DC). The voltage was pre-set to 9 kV and the current to 450 mA. The control reactors were 300 ml beakers closed at the top to prevent introduction of dirt.

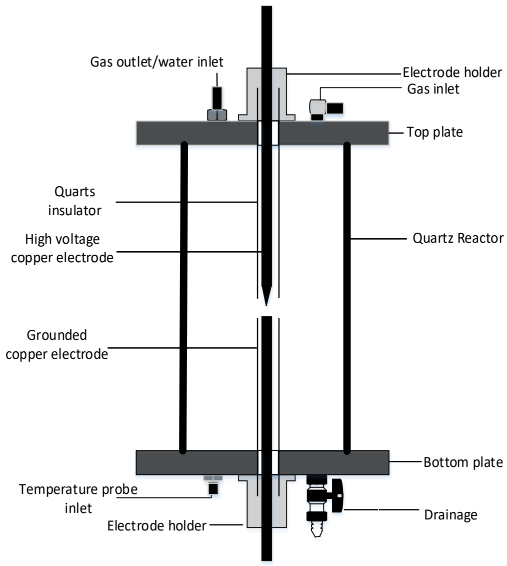


**Fig. 1. Schematic diagram of plasma reactor**

### 2.2 Bacterial preparation, plating and experimental methods

*Escherichia coli* was used as a model in the inactivation experiments. *E. coli* (ATCC 25922) was inoculated into Luria-Bertani (LB) broth and incubated at 37°C for 12–16 h in a shaking incubator (150 rpm) to obtain the bacterial culture used for this study. Sterile distilled water was added to a measured volume of bacterial culture in their log phase (30 and 60 ml) to make up a total volume of 300 ml and subsequently treated in the plasma reactor for various periods of time. The inoculation and every other aspect of the experiment were carried out aseptically. The starting CFU/ml of each control sample was determined by plating about 100 µL of the distilled water-bacterial culture suspension on Coliform ChromoSelect Agar (Sigma-Aldrich) before exposure to plasma treatment. At the end of plasma treatment of each bacteria-distilled water suspension for different time duration (Table 2), about 100 µL of the suspension was removed and spread on Coliform ChromoSelect Agar (Sigma-Aldrich) in a standard Petri dish (90 mm in diameter), and incubated at 37 °C for about 24 h. After which the colony count was done and recorded.

Table 1 summarises the experimental conditions for each of the six experimental batches conducted. Batch 1 and Batch 4 were conducted in semi batch mode with sampling being done every 30 seconds, the total run time was 180 seconds. Only samples in Batch 1 and Batch 4 were exposed to plasma. The control experiments were conducted in batch mode. The total run time for the control experiments was 300 seconds. The samples extracted then analysed as stated in section 2.2.

**Table 1: Experimental procedure description**

| Batch | Procedure Description |
|---|---|
| 1 | 39 600 cfu/ml biological sample exposed to plasma. |
| 2 | 39 600 cfu/ml biological sample is control 1 for experiment. |
| 3 | 39 600 cfu/ml biological sample is mixed with a copper solution of concentration of 20 µg/L. This sample is control 2 for experiment 1. |
| 4 | 130 000 cfu/ml biological sample exposed to plasma treatment. |
| 5 | 130 000 cfu/ml biological sample is control one 1 for experiment 4. |
| 6 | 130 000 cfu/ml biological sample is mixed with a copper solution to a final copper solution of 40 µg/L. This sample is control 2 for experiment 4. |

### 3. Results and discussion

Table 2 shows the reduction in the numbers of cfu/ml units as a function of time. For initial cfu/ml of 39600 and 130000, at 60 and 90 seconds respectively the counts showed a negative result meaning there was total inactivation. Fig. 2 give a graphical representation of the results presented in Table 2.

Table 3 shows the inactivation of the *Escherichia coli* in the control reactors. It can be noted that after 300 seconds exposure to copper salts in solution, the control batches showed less inactivation as compared to the samples exposed to plasma. For initial cfu/ml of 39600 at 30 seconds, plasma had 4.5 log reduction while for samples with 20 µg/L copper, a 1.7 log reduction was registered after 300 seconds. For initial cfu/ml of 1300 00 at 60 seconds, plasma had 3.4 log reduction while for samples with 40 µg/L copper a 0.8 log reduction was registered after 300 seconds.

**Table 2: Bacterial reduction in terms of cfu/ml and log reduction as a function of treatment time**

| Time (s) | Batch 1 Bacterial reduction | | Batch 4 Bacterial reduction | |
|---|---|---|---|---|
| | CFU/ml | Log Reduction | CFU/ml | Log Reduction |
| 0 | 39600 | | 130000 | |
| 30 | 1 | 4.5 | 76 | 3.2 |
| 60 | - | - | 49 | 3.4 |
| 90 | - | - | - | - |
| 120 | - | - | - | - |
| 150 | - | - | - | - |
| 180 | - | - | - | - |

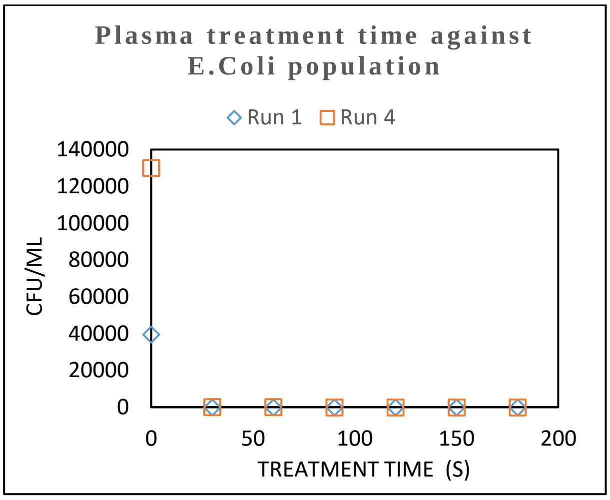


**Fig. 2. Reduction of E.coli colony forming units with increase in plasma treatment time**

The antimicrobial properties of copper were confirmed [5] by the reduction of *Escherichia coli* colony forming unites in Batch 3 and Batch 6 (**Table 3**). The use of copper as electrodes results in enhanced bacterial inactivation effects as they releases copper ions and copper nanoparticles into the water sample being treated. It is has been shown by science that copper ions released from copper surface is capable of collapsing the outer cell membrane of the bacteria by potentially creating a short circuiting of the micro-current in the cell or perhaps as a result of "*oxidative damage*" if the copper ions are released in the presence of oxygen. However, the extent of damage is placed at maximum of 3-log reduction for a time of 2 hours on a copper surface. Nonetheless, the interaction mechanism of copper ions with bacteria is a complex phenomenon.

**Table 3. Percentage and log reduction in E.coli inactivation using copper salt without plasma**

| Batch | Log Reduction | Percent Reduction |
|---|---|---|
| 2 | 0 | 0 |
| 3 | 1.7 | 98 |
| 5 | 0 | 0 |
| 6 | 0.8 | 85 |

**Table 4. Change in pH, Temperature ($^{O}C$) and electrical conductivity (µS/cm)**

| Batch | pH | | Temperature | | Conductivity | |
|---|---|---|---|---|---|---|
| | Initial | Final | Initial | Final | Initial | Final |
| 1 | 7.1 | 5.3 | 27 | 40 | 0.06 | 0.34 |
| 4 | 7.1 | 5.1 | 27 | 38 | 0.06 | 0.29 |

Table 4 illustrates the change in water properties after 180 seconds of plasma treatment. Experimental Batch 1 showed a higher final pH, temperature and conductivity than Batch 4. This can be attributed to a more viscous sample in Batch 4. The arc in Batch 1 was sustained from the start, while for Batch 4 it only became sustained after 20 seconds. This would result in high temperatures and electrode wear, as shown by higher conductivity, in Batch 1. The conductivity and pH were measured after each experimental run using the EC 215 conductivity meter and pH 212 Microprocessor pH meter, both from Hanna Instruments. The final values for pH, temperature and conductivity are comparable for Batch 1 and Batch 4.

After the two sets of experiment, the remaining spiked wastewater in the reactor was drained, decontaminated in the autoclave, and 10 ml samples were taken and analysed in an inductively coupled plasma atomic emission spectroscopy (ICP-AES) or optical emission spectrometry (OES) to determine the concentration of copper ions that were released into the water during treatment. The total concentration of copper ion present in the solution after the experimental run of batch 1 and batch 4 were 0.66 mg/L and 0.47 mg/L respectively.

## 4. Conclusion

Non-thermal plasma technology has potential as a low temperature disinfection method. The current studies clearly indicate the microbicidial effect of plasma-in-liquid direct discharge. Systematic researches are required to evaluate the relative inactivation of different groups of pathogens by the direct plasma-in-liquid approach utilising copper electrodes. There is on-going work to investigate other water-borne pathogens as earlier mentioned, most especially those microorganisms that are resistant to antibiotics. It is thought that the success of the plasma process in the inactivation of bacteria in the batch reactor in a short treatment time could lead to the future design of a flow process that can be integrated with already existing water disinfection methods especially after the clarifier stage.

## 4. References


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