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Cold plasma Technology for Removal of Endotoxin from Dialysis Water

Shaimaa Fakhri Jasim | Yasamen Raad Humudat✉ | Suadad Awad Kadhim

Environment,Water and Renewable Energy Directorate, Ministry of Science and Technology, Baghdad, Iraq.

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ABSTRACT

Cold plasma has emerged as a powerful energy-efficient and environmentally friendly advanced oxidation technique in recent decades as a non-thermal approach in a wide range of applications. It is a form of plasma that is created at low temperatures and can be used for various applications, including water treatment This study aims to determine the influence of Cold plasma treatment on endotoxin reduction in dialysis water. A lab-scale unit was built to implement the experiments and synthetic water (feed solution) was prepared with a known level of endotoxin (0.48 EU/mL). The test for Limulus amebocyte lysate was used to assess concentrations of endotoxin in treated water. The experimental results showed reduce of endotoxins in the cold plasma treatment. This type of treatment reduced the concentration of endotoxin to 0.17 ± 0.09 EU/ml. The results of the study indicated that this could be an innovation in cold plasma jet fields, with numerous applications in dialysis fluid preparation.

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INTRODUCTION

Water is a vital vehicle in the dialysis clinic for delivering life-saving treatment to patients suffering from varied degrees of renal failure, both acute and chronic (Kiran and Kaushal, 2022). Dialysis fluid, also known as dialysate, is used to remove waste products and excess fluids from the blood during hemodialysis. The quality of the dialysis fluid is crucial for patient safety, and minimizing endotoxin contamination is an important goal (Humudat, 2020). This is due to dialysis requires the use of large volumes of water, which can be a source of bacterial contamination, and the process of dialysis itself can cause damage to the patient's blood vessels, which can allow endotoxins to enter the blood stream (Humudat *et al*., 2022).

Cold plasma has been studied as a potential method for reducing endotoxin levels in the dialysis fluid. Endotoxins are a concern in hemodialysis because they can cause inflammatory reactions and contribute to the development of complications in patients undergoing dialysis treatment (Hashmi and Thakur, 2019). Endotoxins are a mixture of protein and lipopolysaccharide (LPS) molecules that are found in the outer membrane of certain gram-negative bacteria, such *as Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* (Humudat *et al*., 2020). These toxins are released when the bacteria die or divide and can cause a range of harmful effects in humans, including fever, septic shock, and organ failure (Wen et al.,2017). Lipopolysaccharides have molecular weights of 10 - 20 kDa; however, due to their amphiphilic

*Corresponding Author Email: *yasmenraad@yahoo.com*

nature, LPS molecules can form aggregates (100 - 1000 kDa) that are too large to pass through dialysis membranes (Glorieux *et al*.,2012).

Cold plasma, also known as non-thermal or atmospheric plasma, involves the generation of ionized gas that contains various reactive species. In the context of dialysis water treatment, cold plasma can be utilized to eliminate various types of contaminants, including bacteria, viruses, endotoxins, and organic compounds. (Zainal *et al*.,2015). The plasma discharge generates highly reactive species such as ozone, hydroxyl radicals, and reactive oxygen species, which have strong oxidizing and antimicrobial properties (Van Nguyen *et al.,* 2019). The cold plasma treatment is typically carried out in a controlled chamber or reactor where the dialysis water is exposed to the plasma discharge. As the water passes through the plasma region, the reactive species interact with the contaminants, leading to their degradation or inactivation Bourke *et al.,* 2017).

One of the advantages of cold plasma technology is that it operates at room temperature, hence the name "cold" plasma. This feature makes it suitable for applications such as water treatment, as it avoids any potential thermal damage to the treated water (Šimončicová et al., 2019). Additionally, cold plasma treatment is known for its ability to efficiently eliminate a wide range of contaminants without requiring the addition of chemicals (Gururani *et al*., 2021).

The American National Standards Institute, the Association for the Advancement of Medical Instrumentation, and the International Organization for Standardization (ANSI/AAMI/ISO) have established maximum allowable concentrations of bacteria and endotoxin in hemodialysis fluids to help prevent pyrogenic reactions and bacteremia in hemodialysis patients caused by bacterial and endotoxin contamination of these fluids (American National Standards Institute,2019). Therefore, the aim of this study was to investigate the efficiency of the treatment of jet-type cold atmospheric plasma for endotoxin inactivation from dialysis

MATERIALS AND METHODS

Water samples were synthesized in Baghdad's Iraqi Ministry of Science and Technology. Limulus amebocyte lysate (LAL) reagents were used to prepare standard endotoxin control (CSE) *E. coli* 500 ng/vial at a concentration of 1,000 EU/ml for use with the gel clot endpoint method, which is engineered to detect the presence of bacterial endotoxins (Wako-pyrostar, 2012).This solution was then mixed with double-pass reverse osmosis RO water with an endotoxin concentration of 0.06 ±0.06 and an electric conductivity of 0.5 S/cm at room temperature to produce synthetic water (feed solution) with an endotoxin content of 0.48 ±0.06 EU/ml that will be used in all experiments. Its endotoxin content is higher than the ANSI/AAMI/ISO dialysis water recommendation, although it is within the range of endotoxin concentration values previously discovered in dialysis water samples collected from various dialysis centers in Iraq (American National Standards Institute, 2019). To eliminate all pyrogen, all glassware was heated for at least 30 minutes at 350 to 400°C (Anderson *et al*., 2003). Certain materials were purchased without pyrogens, such as dilution tubes, micropipette tips, and aluminum caps.

Cold plasma jet experiments design

The preparation of cold plasma for water treatment typically involves the following steps (Bousba et al., 2023): A) Plasma Generation: Cold plasma is typically created by applying a high-voltage electrical discharge to a gas, This discharge ionizes the gas, generating a plasma containing various reactive species. B) Plasma Reactor/Chamber: The generated plasma is then introduced into a dedicated reactor or chamber where the water is treated. The reactor is designed to allow efficient contact between the plasma and the water to maximize the treatment effect. C) Water Treatment: As the water flows through the plasma reactor, it comes into contact with the reactive species present in the plasma.

To apply the atmospheric Cold plasma jet (APJs) for endotoxin detoxification, complete samples of 2ml were taken and placed in sterile containers free of endotoxin of 3ml capacity, and these samples were divided into five groups to assess endotoxin in samples depending on the duration of exposure to the application of APJs time = 0, 3, 5, 10, 15, and 20 minutes) at 0.5 cm and used argon gas) Niemira, 2012(. Variable DC voltage flow rate of air (1 liter/min) (6.5-7.5 KV and 30khz). The sample is then sealed and transferred to the laboratory to be tested for endotoxin by gel clot assay.

Statistical analysis

Statistical analysis was carried out using Microsoft Excel 2010. Quantitative data were expressed as mean ± standard deviation (SD).

RESULTS & DISCUSSION

Cold plasmid jet treatment

Cold plasmid jet treatment was used to disinfect samples of synthetic feed water for the removal of endotoxins at various contact times (0, 3, 5, 10, 15, and 20 min). At the beginning of this experiment, endotoxin concentration was (0.48 ± 0.06 EU/ml), the best contact time to decrease endotoxin concentration to 0.17 ± 0.09 EU/ml was obtained at 20 min. The results were below the limit compared with the ANSI/AAMI international standard endotoxin concentration value (0.25 EU/ml).

This study shows that all the time periods for APJs have reduced endotoxin levels in complete dialysis water samples at the space 0.5 cm when compared with the control sample. The exposure of complete dialysis water samples to APJs at 20 min, was the best time period to reduce the toxin level.

The current study are in agreement with one study proving that cold plasma has the ability to eliminate *E.coli*)Sawangrat *et al*.,2022). Another study suggested that the cold plasma technique has the ability to decontaminate *E.coli* in water (Mentheour and Machala,2022). Moreover, Dahle *et al.* (2013) showed that significant differences between bacterial cells, suggesting that Gram-positive *S. aureus* is less susceptible to plasma treatment than Gram-negative *E. coli* and *P. aeruginosa*.Increasing the exposure time and decreasing the distance decreases the number of cells in the biofilm. However, the combination of close distance and long exposure time resulted in synergistic effects. Also, another study appeared to that cold plasma imposes a great potential for environmental applications, such as water purification and microbial inactivation)Abdel-Wahed *et al*.,2023) This study also corresponds to the research of Bousba et.al. who reported the effect of both treatment time and the introduced ratio of the gas mixture introduced to ignite the plasma discharge (Bousba *et al*.,2022). Moreover, a prior study Kwon *et al.*)2019) demonstrated that water pollutant decontamination following APJs can be achieved within around 12 minutes of treatment.

Table 1. Results for Cold plasma jet treatment for feed water to reduce endotoxin from the initial concentration of (0.48 EU/ml)

Test no.	Control of endotoxin concentration (EU/ml)	Contact time (min)	Endotoxin concentration (EU/ml)
1		0	0.48±0.06
2		3	0.48±0.06
3		5	0.39±0.09
4		10	0.24±0.06
5	0.48±0.06	15	0.24±0.09
6		20	0.17±0.09

It's worth noting that while cold plasma shows promise in dialysis water treatment, further research, and development are necessary to optimize the technology, ensure its long-term reliability, and validate its effectiveness in real-world settings. It's advisable to consult with experts and professionals in the field to get the most up-to-date information on the current state of cold plasma technology in dialysis water treatment.

CONCLUSION

Cold plasmid jet of treatment processes applied in this study has improved the purity of dialysis water and reduced endotoxin levels by degrading. Although the addition of cold plasmid jet instruments increases the investment cost by adding a new step in water treatment, it can help to reduce operating costs by lowering membrane maintenance and extending membrane lifespan through contaminant dispersion. However, more research is necessary to refine the process and validate its efficacy for different applications and settings.

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CONFLICT OF INTEREST

The authors have expressly indicated that there are no conflicts of interest in this paper.

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