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Decoupling locally enhanced electric field treatment (LEEFT) intensity and copper release by applying asymmetric electric pulses for water disinfection

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ABSTRACT

Copper has well-known anti-microbial properties but is typically not considered for drinking water disinfection because of its health risk to human at efficient biocidal concentrations. Locally enhanced electric field treatment (LEEFT) is a cutting-edge technique that aims to inactivate bacteria by generating aqueous pores on the cell membrane through the application of a strong electric field. LEEFT can also increase the permeability of the cell membrane, which promotes the uptake of chemical disinfectants to reduce the required biocidal concentrations. Previously, a coaxial-electrode copper ionization cell (CECIC) was developed to combine copper disinfection with LEEFT, demonstrating superior disinfection efficiency with low effluent copper concentrations (<0.5 mg/L). However, using direct-current (DC) voltages results in a dilemma that a higher voltage is necessary for effective LEEFT disinfection, but a lower voltage is required to limit Cu release. Here, asymmetric electric pulses are employed to decouple the LEEFT intensity from copper release in the CECIC. In this case, LEEFT intensity is primarily determined by the pulse amplitude while the copper release is controlled by the pulse offset. We have demonstrated that the use of asymmetric electric pulses achieves significantly higher inactivation efficiency compared to the DC voltages with the similar level of Cu release. For the water with conductivity similar to tap water (~100 μS/cm), a high inactivation efficiency of 4.7-log is achieved with only 0.49 mg/L copper release. These findings highlight the potential of asymmetric electric pulses as a promising alternative to DC voltages for the practical application of LEEFT-Cu systems in the future.

Introduction

Copper has well-known antimicrobial properties and has attracted more attention as a disinfectant in the past few decades (Arendsen et al., 2019; Grass et al., 2011; Milanino, 2006; Vincent et al., 2016). Copper and copper alloy surfaces are much more microbial-resistant than stainless-steel surfaces, which can be used to reduce the microbial risks in hospitals (Arendsen et al., 2019; Monk et al., 2014; Prado et al., 2013; Salgado et al., 2013; Schmidt et al., 2012). Copper-silver ionization is a promising approach to control *Legionella* outbreaks in hospitals or buildings (Lin et al., 2011; Liu et al., 1998, 1994). Copper ions can also inhibit biofilm formation and eradicate multidrug-resistant bacteria (Portelinha et al., 2021). For water disinfection, a relatively high copper concentration (*e.g.*, > 3 mg/L) is typically required to achieve effective bacteria inactivation (*e.g.*, > 3 log) (Bitton and Vic, 1977; Fowler et al., 2019; Park et al., 2012; Yamamoto et al., 1964). However, excessive ingestion of copper may cause diarrhea, nausea, vomiting, and even liver and kidney damage from long term exposure (Arendsen et al., 2019; Gaetke and Chow, 2003). Because of these adverse health effects, the United States Environmental Protection Agency (USEPA) has set the maximum contaminant level goal (MCLG) of 1.3 mg/L for copper in the National Primary Drinking Water Regulations (USEPA, 1996). Such a low copper concentration is usually not sufficient for bacteria inactivation, and thus copper is usually not applied for drinking water disinfection.

To achieve more effective bacteria inactivation with a lower copper concentration, a promising approach is to integrate copper ionization with locally enhanced electric field treatment (LEEFT). Developed in the past decade, LEEFT uses coaxial configurations and/or nanowire-modified electrodes to enhance the electric field near the electrodes (Huo et al., 2019b; Pi et al., 2021; Wang et al., 2022; Zhou et al., 2020a, 2019). Exposure of bacteria to a strong electric field results in an elevated transmembrane potential, which may cause direct bacteria inactivation or increase the permeability of the cell membrane, facilitating easier entry of chemical disinfectants into the cell (Kotnik et al., 1997, 2015, 2019). In our previous studies, a coaxial-electrode copper

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ionization cell (CECIC) was rationally designed to combine copper disinfection and LEEFT (Zhou et al., 2019, 2020b). In this LEEFT-Cu system, the copper concentration near the center electrode is much higher than that observed in the effluent because the copper ions are electrochemically released from the center electrode, creating a concentration gradient of copper. The area near the center electrode also has higher electric field strength due to the coaxial configuration and attracts bacterial cells due to the effect of electrophoretic and dielectrophoretic forces (Zhou et al., 2019). Attributed to these synergistic effects of copper and LEEFT, superior bacteria inactivation performance (~6-log removal) was achieved with a low voltage of ~1.5 V and effluent copper concentrations less than 0.5 mg/L. (Zhou et al., 2019, 2020b). However, in these studies, direct-current (DC) voltages were applied, which coupled the LEEFT intensity with copper release. Specifically, the LEEFT intensity, *i.e.*, the electric field strength, is proportional to the voltage applied, which also determines the current and thus the copper release. The CECIC has worked well when treating synthetic water with low conductivity (~0.3 μS/cm) prepared from deionized water. However, this coupling effect becomes a problem when the CECIC treats water with higher conductivities that are similar to more practical conditions: with higher conductivity, the system resistance decreases, which increases the current and Cu release; to maintain the designated low Cu release, a lower voltage should be applied, resulting in weaker electric field strength for LEEFT and thus lower antimicrobial efficiency. Therefore, the dilemma between low voltage required to limit Cu release and high voltage for effective electroporation needs to be resolved (Zhou et al., 2020b). For practical applications, it is important to have the capability of tuning the electric field strength while maintaining copper release at a low level.

Here, we propose applying asymmetric electric pulses to decouple the LEEFT intensity and copper release in the CECIC. The asymmetric electric pulses applied in this study are square waves with two main variables, pulse offset and pulse amplitude. Subsequently, we have demonstrated that the copper release is controlled by the pulse offset, while the LEEFT intensity is primarily determined by the pulse amplitude. By decoupling these two parameters, the use of asymmetric electric pulses has achieved a significantly higher inactivation efficiency compared to the DC voltages with the similar level of Cu release. Notably, for the synthetic water with similar conductivity to practical tap water, a superior inactivation efficiency (*i.e.*, 4.7 log) has been achieved with less than 0.5 mg/L copper release.

Results and discussion

Copper release under direct-current (DC) voltages

The copper release in the CECIC is attributed to the electrochemical oxidation of the copper wire, which serves as the anode at the center of the treatment chamber (Figs. 1a and S1). As shown in Fig. 1b and c, the current and copper release increase along with the applied direct-current (DC) voltages. Based on Faraday's laws of electrolysis, the theoretical copper release is proportional to the current flow (Supplementary Note 1). The ratio of actual and theoretical copper release is close to 1 (Supplementary Note 1, Figs. S2 and S3), indicating minimum side reactions on the electrodes and a nearly 100 % columbic efficiency of copper ionization. Notably, as shown in Fig. 1b, when a 1.5 V DC voltage is applied to treat bacteria suspended in deionized water with a conductivity of ~0.3 μS/cm, the corresponding current and copper release are measured at ~0.063 mA and 0.23 mg/L, respectively. A superior inactivation efficiency (*i.e.*, 6-log removal) has been achieved under such conditions in our previous study (Zhou et al., 2019).

In addition to the applied voltages, the current and copper release are influenced by the water conductivity. As shown in Fig. 1c, when the water conductivity increases to ~100 μS/cm (similar to the tap water conductivity), the current and copper release under the same 1.5 V DC voltage surge to 2.5 mA and 11 mg/L, respectively, which are approximately 40 times higher than those observed in the sample prepared with deionized water. Such copper release is unacceptable for drinking water disinfection because of potential health risks (Arendsen et al., 2019; Gaetke and Chow, 2003). To keep the copper concentration below 1.3 mg/L to meet the USEPA standard, only up to 0.4 V DC voltage can be applied to ensure a safe copper release (Fig. 1c). However, this low voltage has been found insufficient to generate an electric field strength capable of achieving high inactivation efficiency, as further discussed in Section 'Water disinfection performance' (Zhou et al., 2020b).

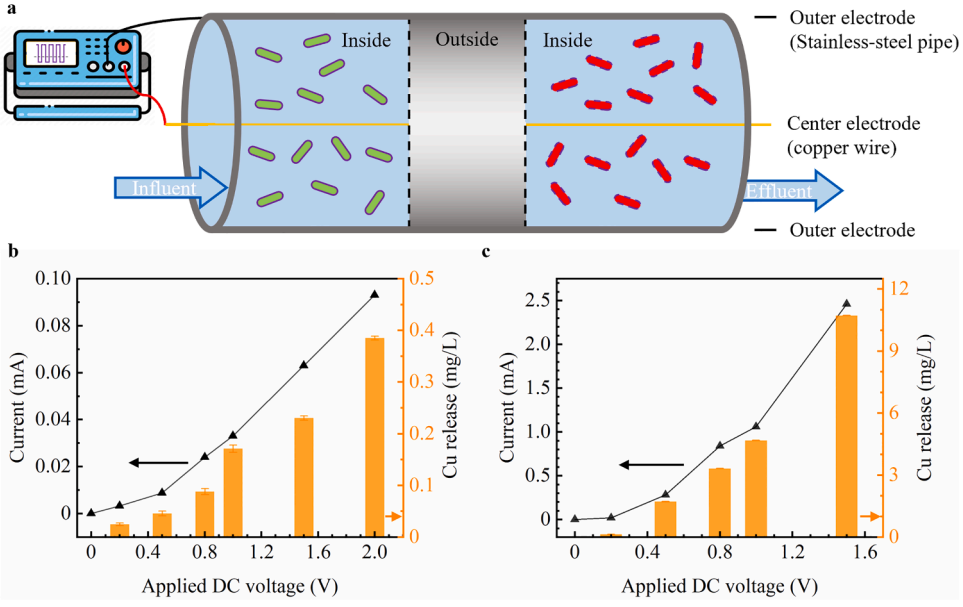

Fig. 1. (a)The schematic of coaxial-electrode copper ionization cell (CECIC). The interior diameter of the stainless-steel pipe and the diameter of the copper wire are 4.88 mm and 255 μm, respectively (*i.e.*, the diameters of the outer and center electrodes). (b, c) Current and copper release at different applied direct-current (DC) voltages when the CECIC is treating bacteria samples suspended in deionized water with a conductivity of ~0.3 μS/cm (b) and a Na_2SO_4 solution with a conductivity of ~100 μS/cm (c). Error bars represent the standard deviations of the triplicate values measured by the atomic absorption spectrometer (AAS).

Copper release under asymmetric electric pulses

Given the challenge of tuning the electric field strength independent of the copper release under DC voltages, we propose employing asymmetric electric pulses to address this problem. The asymmetric electric pulses applied in this study are square waves with different maximum values on the positive and negative sides, which can be considered as the superposition of a DC voltage (at the value of the offset) and symmetric square pulses (with the same amplitude as the asymmetric pulses) (Fig. 2) (Aguilar et al., 2012; Ramaley and Krause, 1969). A high pulse frequency of 1 MHz is maintained in this study to minimize side reactions, such as water electrolysis, because the pulse width (*i.e.*, 0.5 μs) is shorter than the characteristic time of the electrode double layer (typically tens of microseconds) (Luo et al., 2023; Moya, 2018). The absence of water electrolysis also inhibits the generation of reactive oxygen species (ROS), which eliminates the interference of other disinfectant on the inactivation efficiency (Luo et al., 2023). With a fixed frequency, the asymmetric electric pulses can be characterized by two variables, offset (*i.e.*, equivalent DC voltage) and amplitude (Fig. 2).

The copper release under asymmetric electric pulses with different offsets and amplitudes has been tested. As shown in Fig. 3a, when the pulse offset is set to 0.1 V, the copper release under the amplitudes of 1, 3, 5, and 7 V ranges from 0.028 to 0.056 mg/L, which closely resembles the value of 0.041 mg/L observed under 0.1 V DC voltage. The same phenomenon is observed with pulse offsets of 0.2, 0.3, 0.4, and 0.5 V (Fig. 3b–e). By contrast, the copper release increases along with the pulse offset under all amplitudes (Figs. 3f and S4), showing a similar trend as that for DC voltage in Fig. 1. These findings indicate that the copper release is controlled by the pulse offset and independent of the pulse amplitude. In other words, the results can be explained by that the effect from the asymmetric pulses on copper release is also a superposition of that from a DC voltage (at the value of the offset) and symmetric pulses (with the same amplitude as the asymmetric pulses): the copper release is attributed to the equivalent DC voltage, and the high-frequency symmetric pulses have no effect on the electrochemical oxidation of copper as discussed in the previous paragraph. Considering the copper release with 0.5 V offset has already reached 1.1–1.5 mg/L, the maximum pulse offsets of 0.5 V are applied in the bacteria inactivation experiments.

Water disinfection performance

Bacteria inactivation experiments with *E. coli* have been conducted to investigate the disinfection performance of asymmetric electric pulses. As shown in Fig. 4a–c, the log removal efficiencies under both DC voltages and asymmetric electric pulses exhibit an increasing trend with respect to the voltage/offset. Since the electric field strength is at a comparable level under the same amplitude (discussed in Supplementary Note 2, Figs. S6 and S7), the higher inactivation efficiency is likely ascribed to the escalated copper release at higher voltage/offset. In addition, as shown in Fig. 4b and c, the log removal efficiencies with 0 V offset under 3 V and 7 V pulse amplitudes are 0.79 and 0.92 log, respectively. Under these conditions, the copper release is lower than the detection limit (~0.01 mg/L) (Fig. S4). This phenomenon indicates that the electric field still has a slight inactivation effect even without the copper ions, resulting from LEEFT disinfection alone (Jeong et al., 2007; Zhou et al., 2019).

Fig. 4d demonstrates the correlation between the log removal efficiency and copper release and shows that the asymmetric electric pulses achieve higher inactivation than the DC voltages under the similar copper release level. As shown in the figure, when the copper release ranges from 0.40 to 0.53 mg/L, corresponding to a voltage/offset of 0.3 V (Fig. 3c), asymmetric electric pulses with amplitudes of 3 and 7 V achieve removal efficiencies of 3.1 and 4.7 logs, respectively, whereas the DC voltage only reaches a removal efficiency of 2.2 logs. Notably, there is only 0.49 mg/L copper release under the asymmetric electric pulse with 0.3 V offset and 7 V amplitude (Fig. 3c), which is well below the MCLG and applicable for drinking water disinfection and can be assumed as the optimal conditions. In addition, within the copper release ranging from 0.4 to 1.3 mg/L, the asymmetric electric pulses with the amplitude of 7 V achieve a more than 2-log enhancement of inactivation efficiency compared to the DC voltage under the similar copper release. Considering the relationship between log removal efficiency and copper release is almost linear within the copper release below 0.6 mg/L for both the DC voltages and asymmetric electric pulses, the efficacy of copper disinfection can be estimated by calculating the slopes. The results are 3.9, 5.6, and 7.9 logs removal for every mg/L of Cu release for the DC voltages and asymmetric electric pulses with amplitudes of 3 and 7 V, respectively (Fig. S5). Therefore, asymmetric electric pulses demonstrate remarkable performance advantages over the DC voltages, indicating that the stronger electric field induced by a higher amplitude causes the higher permeability of the cell membranes to copper ions and facilitates uptake of copper ions by bacteria, thus enhancing the copper disinfection (Kotnik et al., 2015, 2019; Zhou et al., 2019).

Fig. 4d also shows the DC voltages have a higher log removal efficiency than the Cu control condition without the electric field. This result is probably attributed to in-situ ionization in the CECIC, which was explored in our previous study (Supplementary Note 3, Fig. S8) (Zhou et al., 2019). In-situ ionization establishes a concentration gradient of copper ions along the distance to the center electrode, which increases with rising voltage or pulse offset. This phenomenon also elucidates the progressive enhancement in inactivation of the DC voltage compared to the Cu control condition. It is worth mentioning that at low level of copper release (*i.e.*, < 0.60 mg/L) where the concentration gradient of copper ions is not apparent, the log removal efficiency of the DC voltage is only slightly higher than the Cu control condition (*i.e.*, < 1-log), indicating the electric field induced by the DC

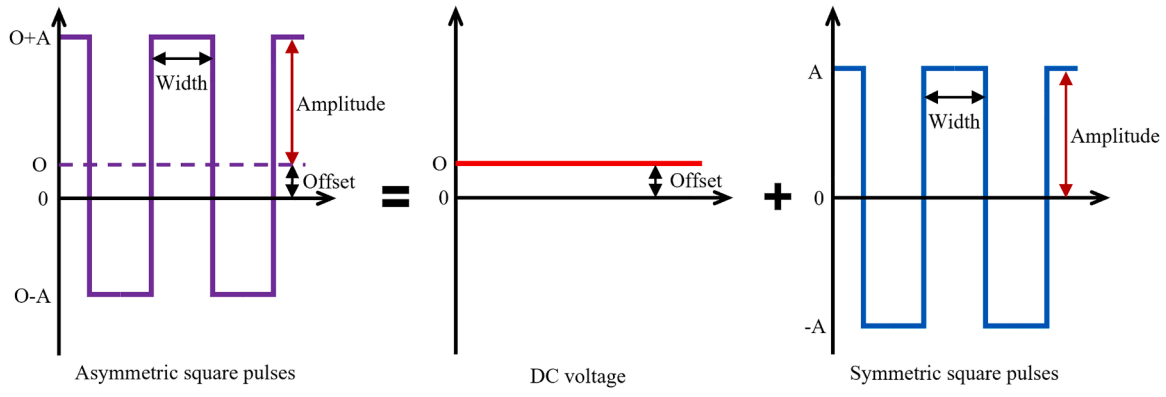

Fig. 2. The schematic of asymmetric electric pulses.

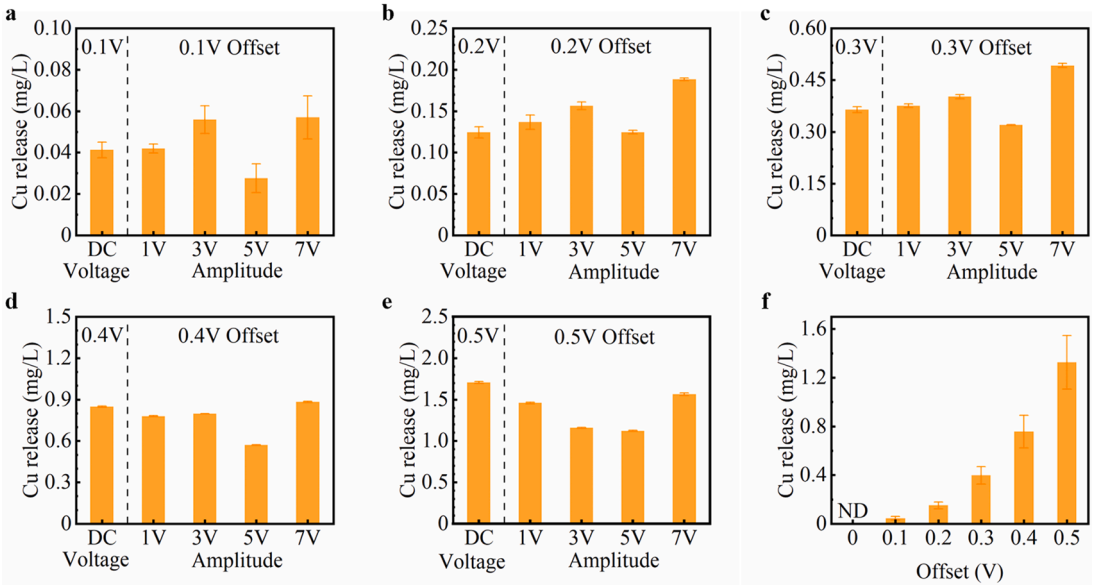

Fig. 3. (a–e) Copper release when treating water samples with a conductivity of ~100 μS/cm under asymmetric electric pulses with different amplitudes at the pulse offsets of 0.1 V (a), 0.2 V (b), 0.3 V (c), 0.4 V (d), and 0.5 V (e), compared with that under equivalent DC voltages. Error bars represent the standard deviations of the triplicate values measured by the atomic absorption spectrometer (AAS). (f) The average of copper release with different amplitudes *versus* the pulse offset. Error bar represents the standard deviation of the copper release with different amplitudes. ND means nondetectable.

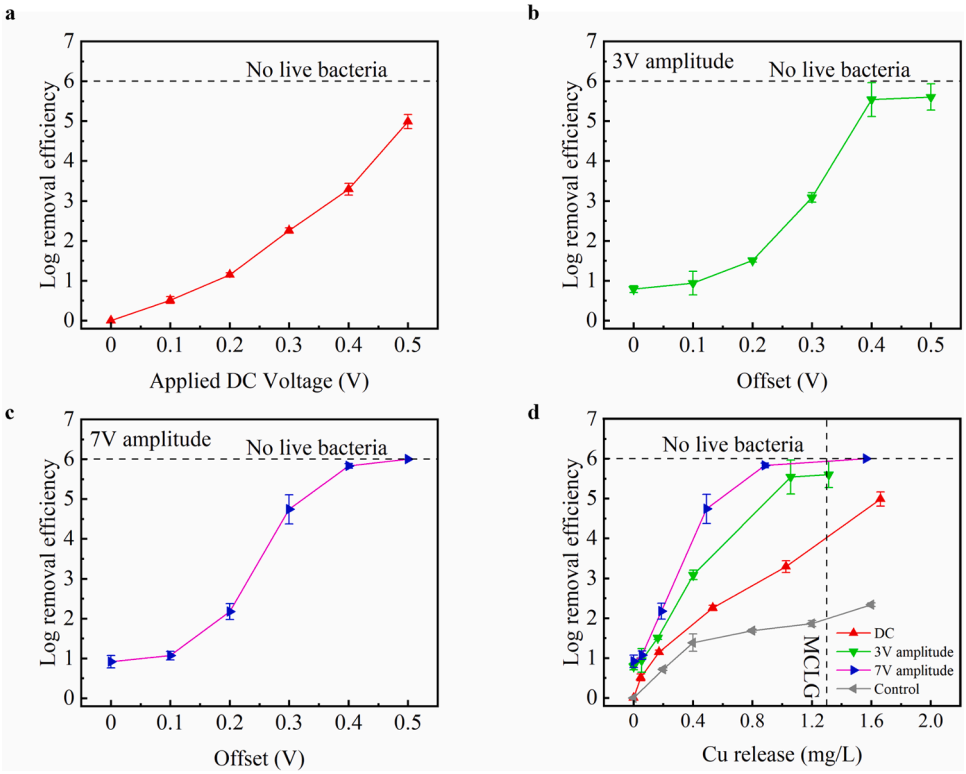

Fig. 4. Water disinfection performance. The conductivity of the water sample is ~100 μS/cm. (a-c) Log removal efficiency of *E. coli* by the CECIC operating at DC voltages (a), asymmetric pulses with 3 V amplitude (b), and asymmetric pulses with 7 V amplitude (c). (d) Log removal efficiency *versus* copper release with DC voltages and asymmetric pulses at 3 and 7 V amplitudes. The control group is administered a $CuSO_4$ solution without the application of DC voltages or asymmetric electric pulses.

voltage is relatively weak and has minimal effect on altering the permeability of the cell membrane.

Prospects and challenges

To assess the cost implications of using asymmetric electric pulses, we selected the optimal conditions detailed in Section 'Water disinfection performance' and estimated the corresponding energy consumption

and electricity cost, which amounted to 124 J/L and about ¢0.6 per year per person (Supplementary Note 4, Table S2). Furthermore, all the components used in the CECIC are inexpensive (*i.e.*, less than $10 for current bench scale prototype) and commercially available. Therefore, we believe the CECIC powered by asymmetric electric pulses can be a cost-effective technique for drinking water disinfection.

In addition to the low energy consumption of the asymmetric electric pulses, our LEEFT-Cu system also shows the advantages over other electrochemical disinfection techniques. In conventional electrochemical disinfection processes, the inactivation of bacteria relies on the generation of active species, such as Cl_2, OCl^-, ·OH, and H_2O_2 (Bergmann, 2021; Hand and Cusick, 2021; Zhang et al., 2023). These active species have relatively high redox potentials (Table S3), which typically necessitate the use of a high voltage that can lead to water splitting and other unintended side electrochemical reactions. However, in this study, the redox potential of Cu/Cu^{2+} is lower than that of the conventional active species (Table S3). Consequently, only a low equivalent DC voltage (*i.e.*, pulse offset) is required, thus circumventing the issues associated with water splitting and side reactions.

Moving forward, our future research remains encumbered by some challenges. First, the water quality characteristics may influence the inactivation efficiency. For instance, the dissolved organic matter (DOM) in nature water may combine with copper and form copper complexes, inhibiting the cellular uptake of copper (Li et al., 2023; Lu and Allen, 2002). Second, the inactivation efficiency of other bacteria may be further explored. The distinct membrane structures of gram-positive bacteria (*e.g., Bacillus, Staphylococcus*) and gram-negative bacteria (*e.g., Escherichia, Legionella*) may yield varying responses to the electric field, necessitating further exploration (Huo et al., 2019a; Wang and Xie, 2023). Additionally, new power sources capable of providing higher amplitude voltage or with higher energy efficiency need to be explored or customized. A more powerful and versatile power source would greatly contribute to our investigations, which will provide valuable insights into optimizing the efficacy of asymmetric electric pulses and facilitating the scalability of the LEEFT-Cu technology for practical application.

Conclusions

To summarize, we have successfully decoupled the LEEFT intensity and copper release in the CECIC through the application of asymmetric electric pulses. The copper release is demonstrated to be controlled by the pulse offset and is independent of the pulse amplitude, while the LEEFT intensity is primarily determined by the pulse amplitude. Compared to the DC voltages, asymmetric electric pulses achieve a superior enhancement in inactivation efficiency and improve the copper disinfection efficacy. Notably, for the water samples with a conductivity of ~100 μS/cm, we achieve a high inactivation efficiency of 4.7-log, accompanied by a minimal copper release of only 0.49 mg/L, well below the MCLG for copper. With the capability of tuning the LEEFT intensity independent of the copper release, the application of asymmetric electric pulses represents a significant advancement towards the practical implementation of the LEEFT-Cu technology. Considering the low cost and the requirement of a lower equivalent DC voltage compared to other electrochemical disinfection methods, our LEEFT-Cu system can be regarded as a highly promising technique for drinking water treatment.

Materials and methods

Chemicals and materials

LB broth (Miller, cat# 97064–114) and LB agar (Miller, Culgene™, cat# 89405–562) were purchased from VWR for the preparation of the bacteria growth and culture. Sodium bicarbonate ($NaHCO_3$, cat# BDH9280–500 G), and sodium sulfate (Na_2SO_4, cat# 97062–438) were also purchased from VWR for use in the experimental procedures. Nitric acid (HNO_3, 70 %, cat# 225711) was purchased from Sigma-Aldrich for AAS measurement. Copper sulfate ($CuSO_4$, cat# 33308–36) was purchased from Alfa Aesar. DI water (~0.3 μS/cm) was collected from a Thermo Scientific Barnstead Nanopure system for all the experiments. *E. coli* 10798 was purchased from ATCC and was used as model bacteria for the inactivation experiments. Copper wires (bare copper wire, AWG30) were purchased from Arcor Electronics for the construction of the electrode.

Fabrication of the CECIC

The coaxial-electrode copper ionization cell (CECIC) was used as the disinfection device, which was made of a commercially available stainless-steel tube positioned in the middle, flanked by two acrylic modules with plugs on both ends. The stainless-steel tube served as the outer electrode, while the copper wire suspended within the cylinder served as the center electrode. Two electrodes were connected to the power source to facilitate the ionization process. The copper wire was passed through the plugs and stuck tightly to avoid a short circuit. The diameters of the outer and center electrodes were 4.88 mm and 255 μm, respectively (*i.e.*, the interior diameter of the pipe and the diameter of the copper wire). The length of the cylinder was 24.5 cm, and the effective volume of the reactor was 4.6 mL.

Generation of DC voltage and asymmetric electric pulse

The DC voltage was generated by a power source, Keithley 2400 Sourcemeter. The current during operation was recorded directly by the power source. The asymmetric electric pulses were in the form of square waves and generated by a waveform generator, Keysight 33522B The pulse frequency and the duty cycle in this study were controlled at 1 MHz and 50 %, respectively. The pulses offsets ranged from 0 V to 0.5 V, while the pulse amplitudes ranged from 1 V to 7 V. An oscilloscope, Tektronix DPO 3032, was used to monitor the actual condition of the asymmetric electric pulses.

Water sample preparation

To adjust the DI water samples to a neutral pH (approximately 7), sodium bicarbonate ($NaHCO_3$) was initially added. Subsequently, sodium sulfate (Na_2SO_4) was introduced to the neutral water samples to achieve an electrical conductivity of approximately 100 μS/cm. The electrical conductivity of the water samples was measured using a Thermo Scientific advanced electrochemical meter (Orion VersaStar Pro).

E. coli was cultured aerobically in LB broth at 35 ℃ for 14–16 h, resulting in a bacterial concentration of approximately 10^9 CFU/mL. The bacterial solution was then centrifuged at 4000 rpm for 5 min using a VWR High Speed Microcentrifuge. Following centrifugation, the bacterial cells were washed three times with DI water to eliminate the effect of the broth medium. Subsequently, the bacterial solution was diluted 100-fold using the prepared water samples to serve as the influent for the experiments. The parameters of the influent are shown in Table S1.

Bacteria inactivation experiments

The setup of the CECIC system is shown in Fig. S1. In this study, the flow rate was maintained at 4.6 mL/min (*i.e.*, 1 min HRT) using a peristaltic pump (MasterFlex L/S). The standard plate count method was used to measure the bacterial concentration in the influent and effluent (Sanders, 2012). To ensure the complete uptake of copper by the bacteria, the effluent was allowed to sit for 2 h before plating. The agar plates were incubated overnight at a temperature of 35 ℃ to allow for visible colony formation and subsequent counting. The log inactivation efficiency was calculated from the influent and effluent concentration.

To conduct the copper control experiments, a series of copper sulfate ($CuSO_4$) solutions with concentrations ranging from 0 to 1600 μg/L were prepared. Bacteria were inoculated into these solutions, which were subsequently placed on a New Brunswick Scientific platform shaker (InnovaTM 2100) for 2 h to facilitate bacterial interaction with the copper ions before sample collection, which was consistent with the LEEFT-Cu experiments.

Copper concentrations were measured using Atomic Absorption Spectrometer (PerkinElmer PinnAAcle 900F, with PerkinElmer S10 Autosampler) according to USEPA method 7000B (USEPA, 2007).

CRediT authorship contribution statement

Feiyang Mo: Investigation, Data curation, Formal analysis, Validation, Writing – original draft. **Jianfeng Zhou:** Conceptualization, Methodology, Supervision, Writing – review & editing. **Cecilia Yu:** Investigation, Data curation, Validation. **Feifei Liu:** Software, Visualization. **Manhitha Jumili:** Investigation, Data curation, Validation. **Yuxiao Wu:** Investigation, Data curation, Validation. **Xing Xie:** Conceptualization, Project administration, Funding acquisition, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.wroa.2023.100206.

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