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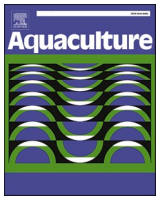

Rapid and safe electrochemical disinfection of salt water using laser-induced graphene electrodes

Ju Zhang [a], Liqing Huang [a], Pok Him Ng [a], Ladan Jahangiri [a], Qianjun Huang [a], Libei Huang [b], Brett MacKinnon [a], Ying Yang [a], Omid Nekouei [a], Yefeng Yang [a], Andrew Ferguson [c], Ana Rita Marques [a], Ruquan Ye [b,*], Sophie St-Hilaire [a,*]

[a] Department of Infectious Diseases and Public Health, Jockey Club College of Veterinary Medicine and Life Sciences, City University of Hong Kong, Hong Kong, China
[b] Department of Chemistry, State Key Laboratory of Marine Pollution, City University of Hong Kong, China
[c] Veterinary Diagnostic Laboratory, Jockey Club College of Veterinary Medicine and Life Sciences, City University of Hong Kong, Hong Kong, China

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ABSTRACT

Bacterial infections account for one of the major causes of disease losses in aquaculture. Antibiotics are the most common method used to mitigate these infections, but over the last few decades this has given rise to antimicrobial resistance so finding alternatives to these treatments is imperative. Here we report a drug-free cost-effective method for rapid and safe water disinfection. A pair of laser-induced graphene (LIG) electrodes charged with low voltage (2 V) rapidly inactivated *Escherichia coli* and *Bacillus subtilis* in various circulating saltwater systems without significant changes in water quality parameters (pH, dissolved oxygen, and temperature). Bacterial inactivation was enhanced with increasing water salinity during electrochemical disinfection using LIG electrodes. Meanwhile, the concentrations of oxidants such as hydrogen peroxide and ozone were generally low regardless of water salinities, and chlorine was not detectable during the treatments. No health impacts were observed in Japanese medaka exposed to eight days of 1 h LIG electrochemical treatments applied twice a day. Our findings suggest that rapid disinfection of saltwater could be achieved using LIG electrodes without negative health impacts on fish, providing potentially an efficient and safe method for controlling bacteria in saltwater systems.

1. Introduction

Aquaculture, including brackish and marine water aquaculture, has been expanding globally to meet the increasing food demand (Costello et al., 2020; Edwards et al., 2019). However, a number of emerging fish diseases, including those of bacterial origin, have limited the growth of the sector (Rivas et al., 2013; Stentiford et al., 2022). Traditional methods for controlling bacterial diseases in aquaculture include ozonation (Mecha et al., 2017), ultraviolet radiation (Yang et al., 2016), and chlorination/dichlorination (Liu et al., 2018). However, these approaches have limitations, such as high cost, the need for sophisticated equipment, or incomplete elimination of pathogens (Chuah et al., 2016; Grob and Pollet, 2016). Some disinfection methods are not ideal for saltwater systems, particularly because they produce toxic by-products that can be lethal to fish (Guilherme et al., 2019; Hu et al., 2018; Ikehata, 2019).

In recent years, electrochemical disinfection has emerged as a novel, next-generation technology for the inactivation of microorganisms in medical environments, the food processing industry, and water treatment plants (Fan et al., 2018; Lacasa et al., 2019; Martínez-Huitle and Brillas, 2021). Electrode materials used in these systems can profoundly affect the disinfection potential of the technology. Electrochemical disinfection using electrode materials such as platinum, zirconium dioxide, titanium dioxide, and boron-doped diamond electrodes exhibit excellent microorganism inactivation during wastewater treatment, but they are expensive and require high energy input (Bruguera-Casamada et al., 2017; Dbira et al., 2019; Demirel et al., 2018; Isidro et al., 2020; Reddy et al., 2019). In addition to the high investment costs, some of these electrode materials produce high levels of oxidants such as chlorine and toxic by-products during electrochemical treatment, which can be detrimental to aquatic organisms (Periyasamy et al., 2022; Schaefer et al., 2015). This is particularly an issue for saltwater treatment because

* Corresponding authors.
E-mail addresses: ruquanye@cityu.edu.hk (R. Ye), ssthilai@cityu.edu.hk (S. St-Hilaire).

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the high concentration of sodium chloride in saltwater can act as a source of chlorine during electrochemical disinfection (Ben-Asher and Lahav, 2019; Serna-Galvis et al., 2017). And therefore, effective and safe use of electrochemical disinfection in saltwater systems is urgently needed to find an electrode material that is relatively inexpensive and does not produce high levels of oxidants or other toxic by-products during the treatment process.

Graphene has recently been highlighted as a potential candidate for electrochemical water treatments as it is non-toxic, conducts electricity efficiently, and has good mechanical strength (Ali et al., 2018; Azhdari et al., 2019; Li et al., 2019). The ease of creating laser-induced graphene (LIG) from a wide variety of carbonaceous materials at a relatively low cost also makes this material potentially suitable for large-scale water treatment applications (Barbhuiya et al., 2021; Huang et al., 2020; Zhu et al., 2019). In addition, LIG electrodes were shown to inactivate pathogens rapidly (Singh et al., 2017). The mechanism of action for the disinfection properties of graphene is not well described but has been at least partially attributed to the physical and electrical contact of the bacterial cells with the LIG surfaces (Singh et al., 2017). Nonetheless, there is limited information on the use of LIG to control bacterial pathogens in aquaculture saltwater systems and its impact on fish health. In this study, we investigated the antibacterial properties of LIG electrodes against model Gram-negative (*Escherichia coli*) and Gram-positive (*Bacillus subtilis*) bacteria in water with different salinities. We also varied the voltage applied to the LIG electrodes and assessed different water parameters, including common oxidants (hydrogen peroxide, dissolved ozone, and chlorine) generated during the electrochemical treatments. To further evaluate the safety of using this technology in saltwater systems, we also conducted a toxicity assessment on Japanese medaka (*Oryzias latipes*) using periodic treatments with LIG electrodes and 3 V of electricity.

2. Materials and methods

2.1. Bacterial preparation

The disinfection properties of LIG electrodes were evaluated on two model lab-safe organisms: *Escherichia coli* and *Bacillus subtilis* as representative Gram-negative and Gram-positive bacteria, respectively. Briefly, a single colony of these bacteria cultured on a tryptic soy agar (TSA) (Sigma-Aldrich, USA) plate was transferred to 1 mL of tryptic soy broth (TSB) (Sigma-Aldrich, USA) and incubated overnight on a shaking plate (250 rpm) in an incubator at 37 °C. After 24 h, we transferred 100 μL of this bacterial solution into 9.9 mL TSB and harvested the solution after 4 h (mid-exponential growth phase). Cultures were then centrifuged at 5000 rpm for 15 min, and cells were washed three times with sterilized PBS (Sigma-Aldrich, USA) to remove residual media. The bacterial cells were diluted with PBS to an optical density of 0.4–0.45 for *E. coli* and 0.75–0.8 for *B. subtilis* at 600 nm to achieve a bacterial concentration of approximately 10^8 CFU/mL.

2.2. Fabrication of LIG and disinfection filtration system

A polyimide (PI) film (Zeman Tape Material Technology, China) with a thickness of 0.05 mm was irradiated with a 10.6 μm CO_2 laser marking machine (MS-1380, Min Sheng Laser Co Ltd., China) to make LIG. The laser power, speed, and line spacing were set as 1.8 W, 500 mm/s, and 700 mm, respectively. The LIG membranes were cut into two 2 cm × 8 cm strips and placed 2 mm apart in an external filtering box (PF-120, Shiruba, Taiwan, China) with a cotton filter (230A, XILONG, Guangdong, China) supporting the LIG membranes. The LIG membranes were suspended 5 cm below and 3 cm above the water line. A direct current power source (UTP1306S, Uni Trend Technology China Co Ltd., China) was connected to the LIG segment above the waterline. The voltage applied to the LIG varied depending on the experiment. Water was recirculated in the aquarium after it passed over the LIG strips at a flow rate of 120 L/h. We used 2 L aquariums to assess the antibacterial effects of LIG electrodes and 7 L aquariums to evaluate its impact on fish health (Supplementary Fig. 1).

2.3. Bacterial inactivation by LIG electrodes

Several independent experiments were conducted to assess the antibacterial effects of LIG electrodes using the 2 L disinfection filtration system described above. All experiments were done at room temperature (~20 °C) in triplicates, with the water flow running continuously for the duration of the experiment (i.e., ~ 2 h). Approximately 5×10^5 CFU/mL of bacteria (type of bacteria depended on the experiment) was added to the experimental tanks based on the stock solution concentration of 10^8 CFU/mL. To assess the viability of bacteria in each experiment, 4 mL of water was withdrawn at 0, 5, 30, 60, and 120 min post-initiation of the current on the LIG. Bacteria were quantified on eosin-methylene blue (EMB) medium using the plate count method described in Li et al. (2021).

To assess the difference between the disinfection properties of LIG towards Gram-negative versus Gram-positive bacteria, 2 V of electricity was applied to the tanks' electrodes containing either *E. coli* or *B. subtilis*. To assess the disinfection properties of LIG using different voltages (i.e., 1 V, 2 V, and 3 V), *E. coli* was used as the model bacteria. For these two experiments, the salinity of the water was adjusted to 2.92 parts per thousand (ppt) NaCl. In a third experiment, we assessed the effect of LIG in different water salinities using *E. coli* as our model organism and 3 V of electricity. Three salinities were evaluated in this experiment representing low (0.5 ppt), moderate (3.0 ppt), and high (35 ppt) saline water. Low and high salinities were created using NaCl (Sigma-Aldrich, USA), as well as sea salt (Marinium, Mariscience International Co., Thailand). The moderate saline water was only evaluated using sea salt. Lastly, we evaluated the electrochemical treatment using LIG electrodes in natural seawater collected from a marina in Aberdeen, Hong Kong SAR, China. To evaluate the antibacterial effect of LIG in this type of salt water, we spiked the water with *E. coli* at 5×10^5 CFU/mL and used 3 V of electricity on the LIG electrodes.

2.4. Impact of electrochemical treatment using LIG electrodes on fish health

Japanese medaka (*Oryzias latipes*) (State Key Laboratory in Marine Pollution in the City University of Hong Kong) were used to assess the health impact of electrochemical treatment with LIG electrodes. All animals were treated according to the guidelines released by the European Union EU Directive 2010/63/EU for animal experiments, and all experiments were approved by the City University of Hong Kong Research Committee for their compliance with the Animals (Control of Experiments) Ordinance, Cap.340, of the Hong Kong Special Administrative Region. Fish were acclimatized to the laboratory conditions for two days without food before exposure to the LIG technology. In brief, nine tanks were used for the experiment and each tank was filled with 7 L artificial seawater with the following water quality parameters: dissolved oxygen (DO) ≥6 mg/L, 28 ppt salinity, pH 8.4 ± 0.1, and temperature of 25 ± 2 °C. Fish were provided with a 14-h light:10-h dark cycle (Bo et al., 2011). We added ~5×10^5 CFU/mL of non-pathogenic *E. coli* to each tank daily to assess the disinfection efficiency of the LIG while evaluating the impact of LIG technology on fish health. Ninety one-year-old Japanese medaka were randomly distributed into the tanks. We ran three replicate treatment tanks ($n = 15$ fish per tank). In the treatment tanks, three volts of electricity were applied to the LIG electrodes for 1 h twice a day for eight days. The negative control tanks ($n = 3$) had the same number of fish ($n = 15$ fish per tank) and same concentration of *E. coli* as our treatment tanks and were also exposed to LIG, but no electricity was applied to the electrodes. The positive control tanks ($n = 3$) did not contain fish but were spiked with the same concentration of *E. coli*. No electricity was applied on the LIG electrodes in

the positive control tanks. To ensure the LIG electrodes were actively disinfecting water, we changed the LIG electrodes at day 5 because we observed a slight decline in the disinfection property after day 4.

We monitored fish for abnormal behaviour, including reduced feeding response to the daily feedings (using dry commercial feed from Ecosystems, USA), erratic swimming, and sitting on the bottom of the tank. These were described over the course of the experiment. The number of dead fish was also recorded daily. Survival rates across groups were compared to determine whether exposure to electrochemical treatment using LIG electrodes had any lethal effects on Japanese medaka.

At the end of the experiment, five random fish per tank were collected for histology. These fish were euthanized with MS222 (1 g/L) before being fixed in 10% buffered formalin solution. Histological slides of the gills were stained with haematoxylin and eosin, and examined by light microscopy for evidence of tissue damage by a veterinary pathologist.

2.5. Water quality

We measured water quality (pH, DO, and temperature) in all experiments. Temperature and DO were measured using a YSI probe (YSI ProODO Xylnm, YSI Incorporated, USA). The pH was measured simultaneously using a pH probe (ECO pH^{+}, Trans Instruments (S) Pte Ltd., Japan). Ammonia was only measured in the toxicity assessment on fish by a portable parallel HACH analyser using total ammonia Chemkey® reagents (SL1000 – PPA, Hach, USA). Dissolved ozone was measured in all experiments using a portable dissolved ozone meter (DOZ-30, Dini Purification Equipment Co Ltd., China). Total chlorine and free chlorine were measured in all experiments using HACH test strips (HACH, USA). Hydrogen peroxide production was assessed in all experiments except for the natural seawater experiment using an Amplex™ Red Hydrogen Peroxide/Peroxidase Assay Kit (Thermo Fisher Scientific, USA).

2.6. Statistical analysis

We compared the log-transformed bacterial counts for different treatment groups at each time point using non-parametric Kruskal–Wallis tests because the assumptions of parametric tests were not met. All values were expressed as median values for the triplicate tanks in each treatment with a 95% CI. Differences were considered significant if $p < 0.05$. Median values of the concentration of hydrogen peroxide between treatment groups were also analysed similarly using Kruskal–Wallis tests. All statistical analyses were performed in Stata software (Version 17, Stata Software, Texas, United States).

3. Results

3.1. LIG disinfection of gram-positive and gram-negative bacteria

The electrochemical disinfection of Gram-negative (*E. coli*) and Gram-positive (*B. subtilis*) bacteria was observed after 5 min of exposure to 2 V of electricity (Fig. 1A). For both types of bacteria, there was a significant reduction in counts after 30 min of exposure to the LIG electrodes. Further, there was a 4 to 5 log reduction in both types of bacteria after 120 min of the treatment. Despite having the same concentration at the start of the study, *E. coli* had a significantly lower bacterial count than *B. subtilis* after 30 min of electrochemical treatment ($p = 0.0495$). In addition, complete removal of *E. coli* was observed at 120 min while, on average, 1-log CFU/mL of *B. subtilis* remained viable in the water at this time point (Fig. 1A). Bacteria in untreated controls tanks remained relatively constant and elevated throughout the studies (Fig. 1).

3.2. LIG disinfection using different voltages (1 V, 2 V, and 3 V)

E. coli inactivation was only observed when at least 2 V of electricity was applied to the LIG electrodes, and the effect appeared after 5 min of exposure (Fig. 1B and C). LIG with 1 V had no significant impact on *E. coli*. In contrast, when 3 V was applied to the LIG membranes, there was a significant reduction in *E. coli* relative to both the negative control (0 V) ($p = 0.0463$) and the 1 V treatment group ($p = 0.0463$) (Fig. 1B). A similar inactivation trend was observed when 2 V was used, with a significant reduction in *E. coli,* compared to the control, after 5 min ($p = 0.0495$) (Fig. 1C). There was no significant difference in *E. coli* inactivation between the 2 V and 3 V treatment groups (Fig. 1C).

3.3. LIG disinfection under different water salinities and in natural seawater

The effectiveness of electrochemical disinfection with LIG was correlated with water salinity. As the salinity increased, the effect of this disinfection technology was enhanced (Fig. 2 A-D). When LIG was used in low saline water (i.e., both 0.5 ppt sea salt and 0.5 ppt NaCl), we observed no significant inactivation of *E. coli* compared with the negative control group throughout the experiment (120 min) (Fig. 2A and B). While in moderately saline water (3 ppt sea salt), there was, on average, a 2- to 3-fold log reduction in bacteria after 60 min (Fig. 2C and D). This reduction in bacteria at 60 min and 120 min was significantly different compared to the control group exposed to LIG without electricity ($p = 0.0495$) (Fig. 2C and D). At high salinity (i.e., both 35 ppt NaCl and 35 ppt sea salt), there was an enhanced and more rapid inactivation of bacteria compared to lower salinity water, as well as compared to the 35

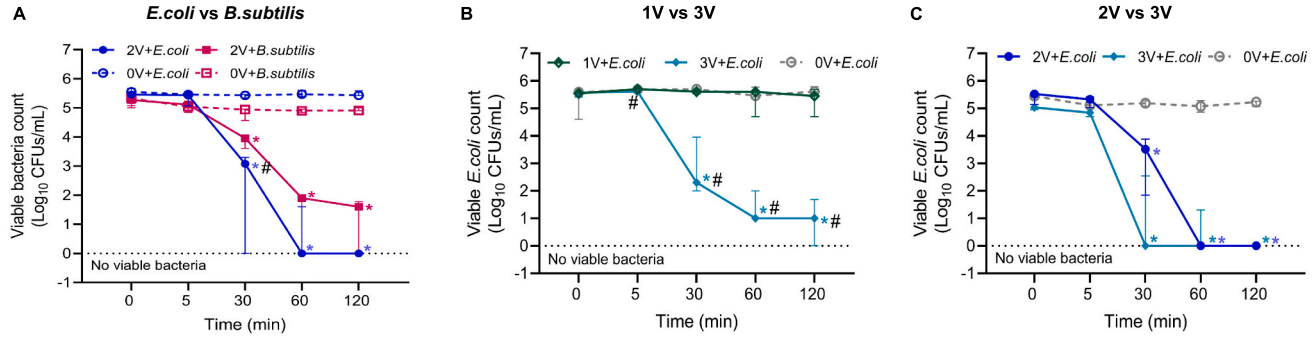

Fig. 1. Inactivation performance by LIG electrodes for different types of bacteria and LIG inactivation at low voltages. A: *E. coli* and *B. subtilis* inactivation by LIG at 2 V; B: *E. coli* inactivation by LIG applied with 1 V and 3 V; C: *E. coli* inactivation by LIG applied with 2 V and 3 V. * represent the significant difference ($p < 0.05$) between the treatment and its corresponding control; #: significant difference ($p < 0.001$) between treatment groups. The median value of three biological replicates is shown, and bars represent the 95% CI ($n = 3$).

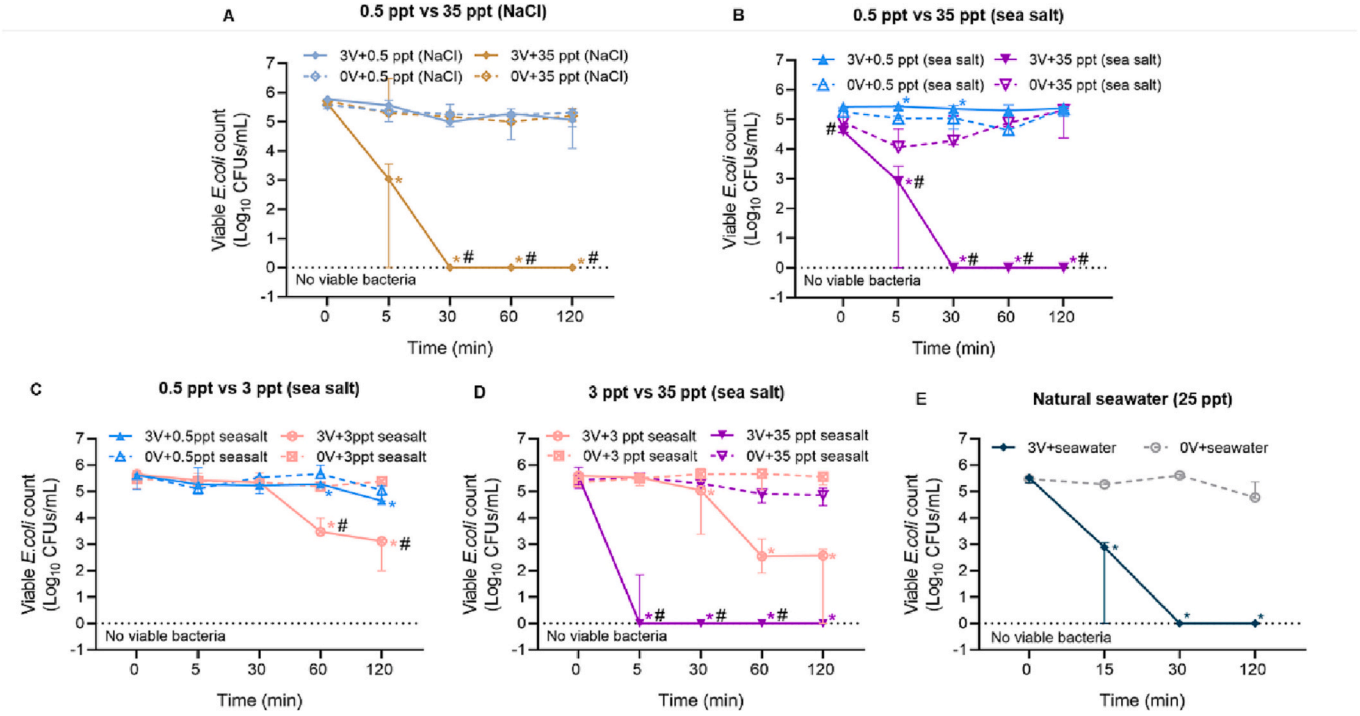

Fig. 2. Bacteria inactivation by LIG electrodes in different water saline environments. A: *E. coli* inactivation by LIG at 3 V in 0.5 ppt NaCl (low saline water) and 35 ppt NaCl (high saline water); B: *E. coli* inactivation by LIG at 3 V in 0.5 ppt sea salt (low saline water) and 35 ppt sea salt (high saline water); C: *E. coli* inactivation by LIG at 3 V in 0.5 ppt sea salt (low saline water) and 3 ppt sea salt (moderately saline water); D. *E. coli* inactivation by LIG at 3 V in 3 ppt sea salt (moderately saline water) and 35 ppt sea salt (high saline water); E. *E. coli* inactivation by LIG at 3 V in natural seawater with a salinity of 25 ppt. * represent the significant difference ($p < 0.05$) between the treatment and its corresponding control; #: significant difference ($p < 0.05$) between treatment groups. The median value of three biological replicates is shown, and bars represent the 95% CI ($n = 3$).

ppt negative control group (i.e., not electricity applied to the LIG membranes) (Fig. 2A, B, and D). After 30 min of exposure to activated LIG, no live bacteria were detected in the treated 35 ppt salinity water tanks. In contrast, there was no change in the untreated 35 ppt control groups in all three experiments (Fig. 2A, B, and D). In the experiment where we used seawater from a marina in Hong Kong, we also observed a rapid decline in the spiked *E. coli* in the treated tanks and no decline in the control tanks (Fig. 2E). After 30 min, we could not detect *E. coli* in the treated natural seawater group (Fig. 2E).

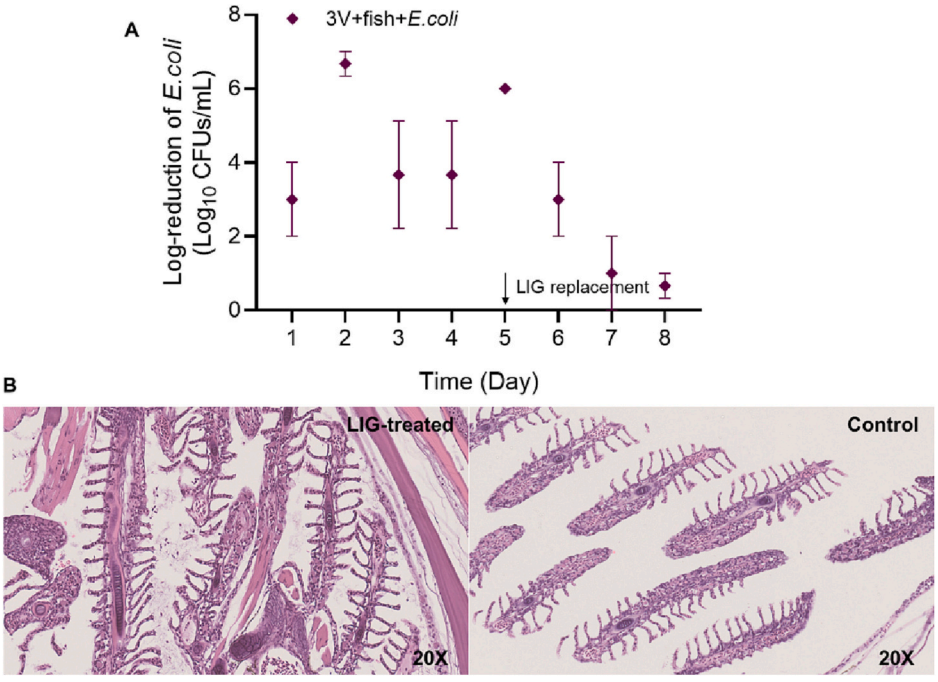

Fig. 3. Antibacterial performance of LIG electrodes and the histology in the fish study. A: The percentage reduction of *E. coli* by LIG electrodes using 3 V of electricity. The mean value of three biological replicates is shown and bars represent the standard error (SEM) ($n = 3$); B: gill histology of the Japanese medaka after 8-day of exposure to periodic electrochemical treatment with LIG electrodes and 3 V of electricity; C: gill histology of the Japanese medaka from after 8-day exposure to LIG electrodes with no electricity.

3.4. Impact of electrochemical treatment using LIG electrodes on fish health

Our LIG electrodes actively disinfected *E.coli* in the fish tanks (Fig. 3A). We did observe a slight decline in the disinfection potential after 7 days despite changing the electrodes on day 5. However, this reduction in disinfection was driven by one pair of electrodes (Fig. 3A). Overall our study suggests fish were successfully exposed to electrochemical treatment trough out the 8-day trial except for one tank which may only have had exposure for 6 days. Our findings indicate that the fish tolerated 3 V of electrochemical treatment with LIG electrodes twice a day with no signs of distress. The fish had no observable behavioural changes and no significant histological changes in the gills (Fig. 3B).

3.5. Water quality

Changes in water parameters (temperature, pH, DO) during the electrochemical treatments were relatively minor. Average pH in the lower salinity tanks ranged from 6.4 to 7.7 (Fig. 4A and B). The pH in high saline water (35 ppt sea salt) was naturally higher, but remained at 8.5–9 for the duration of the study (Fig. 4C). The mean DO was similar between different water conditions (8.40–9.15 mg/L) and remained relatively stable through the experiment (Fig. 4 D—F). There were differences in the initial starting temperatures between groups; however, this difference was never >1.5 °C, and the temperature remained constant within tanks through all the experiments (Fig. 4 G-I). The maximum temperature change within a tank was 1 °C, which only occurred in one experiment (Fig. 4G). For our natural seawater experiment and the fish assessment studies, the water quality parameters (pH, DO, and temperature) had no noticeable changes compared to control tanks (Supplementary Fig. 2 B—D, and Supplementary Fig. 3C-E).

No chlorine was detected in any of the experiments. We could only detect ozone after electrochemical disinfection when the technology was used in high saline water (i.e., both 35 ppt sea salt and 35 ppt NaCl) (Fig. 5 A-C) and natural seawater (Supplementary Fig. 2A). At 120 min, the average concentration of dissolved ozone was approximately. 0.056 mg/mL and 0.030 mg/mL in the LIG-treated high saline water prepared by 35 ppt sea salt and 35 ppt NaCl, respectively. The ozone concentration was slightly higher than this in our natural seawater experiment, with an approximate average of 0.097 mg/L (Supplementary Fig. 2A).

The generation of hydrogen peroxide similarly increased over time in all tanks exposed to LIG with 2 V or higher, except in tanks with high salinity water (Fig. 6 A-G); however, the levels were consistently below 1 mg/L (Fig. 6). There was a slight, but significant difference in the concentration of hydrogen peroxide in tanks treated with 3 V compared to tanks treated with 2 V (Fig. 6C). A significantly higher concentration

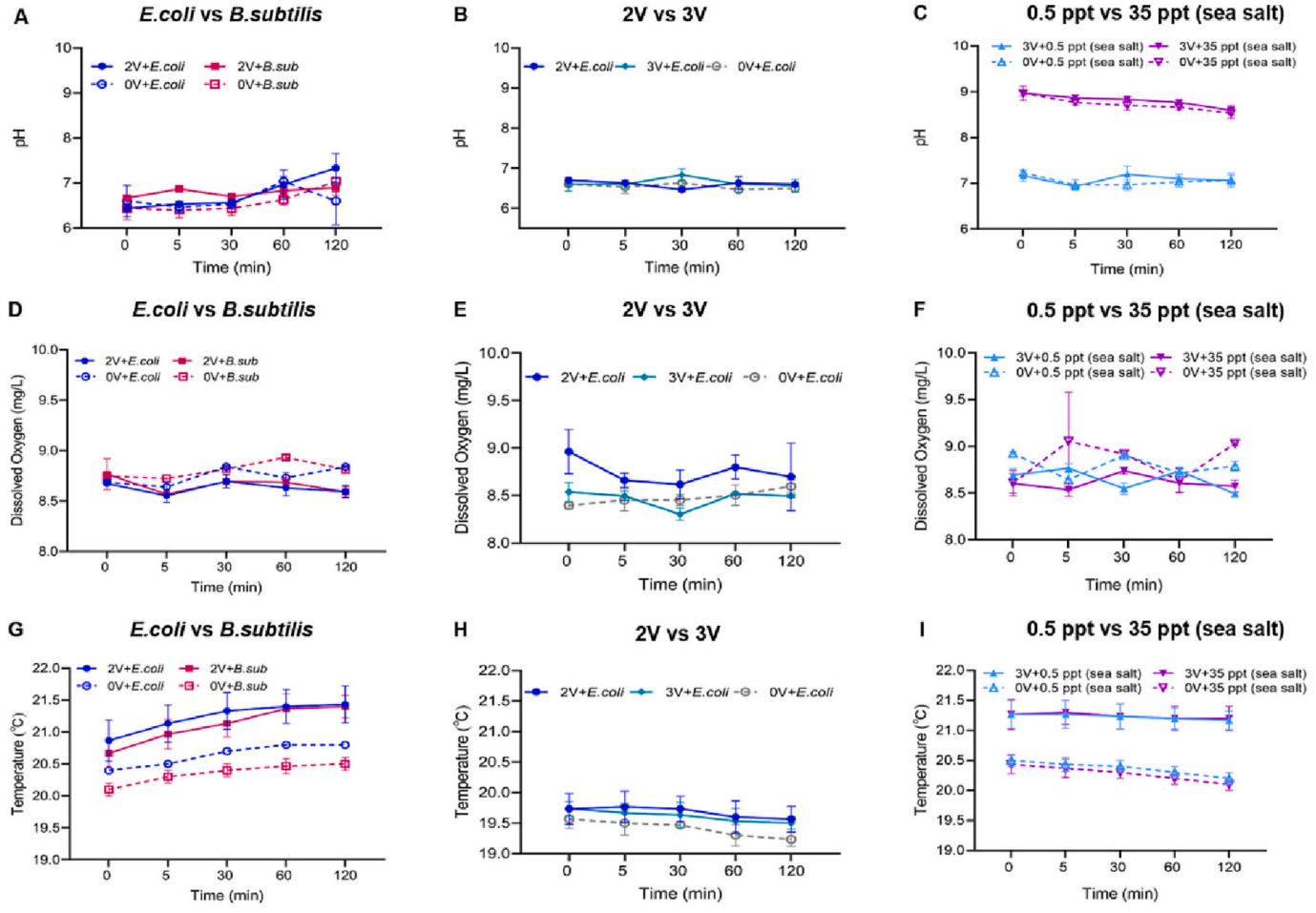

Fig. 4. Water parameters (pH, DO, temperature) during EC treatment. A. pH in the slightly saline water (2.92 ppt NaCl) spiked with different bacterial strains under 2 V; B. pH in the slightly saline water spiked with *E. coli* under 2 V and 3 V; C. pH in the 0.5 ppt sea salt (low saline water) and 35 ppt sea salt (high saline water) spiked with *E. coli* under 3 V; D. DO in the slightly saline water (2.92 ppt NaCl) spiked with different bacterial strains under 2 V; E. DO in the slightly saline water (2.92 ppt NaCl) spiked *E. coli* under 2 V and 3 V; F. DO in the 0.5 ppt sea salt (low saline water), and 35 ppt sea salt (high saline water) spiked with *E. coli* under 3 V; G. Temperature in the slightly saline water (2.92 ppt NaCl) spiked with different bacterial strains under 2 V; H. Temperature in the slightly saline water (2.92 ppt NaCl) spiked *E. coli* under 2 V, and 3 V; I. Temperature in the 0.5 ppt sea salt (low saline water) and 35 ppt sea salt (high saline water) spiked with *E. coli* under 3 V. The mean of three biological replicates is shown, and error bars represent the standard deviation (SD) ($n = 3$).

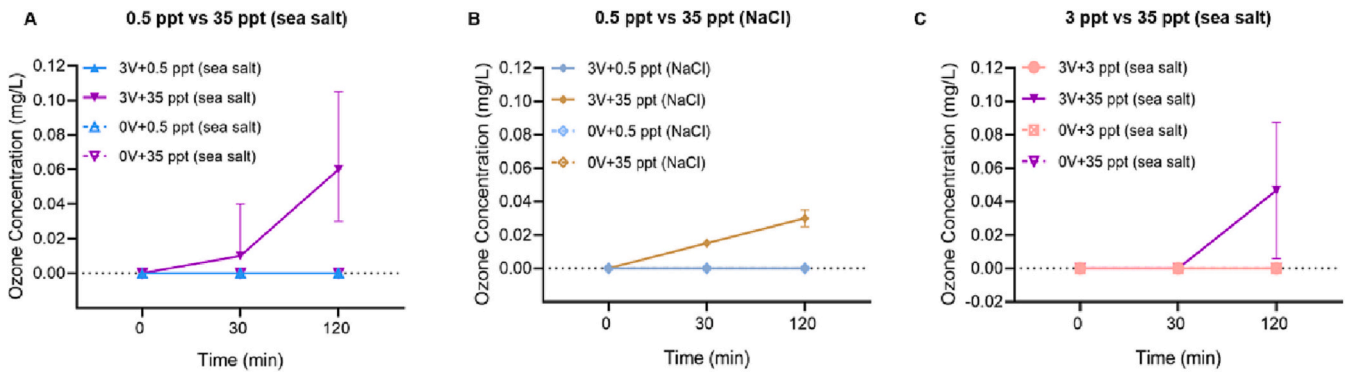

Fig. 5. Dissolved ozone concentration in seawater during three independent electrochemical experiments using LIG electrodes. A. Dissolved ozone concentration measured in tanks with LIG electrodes applied at 3 V in 0.5 ppt sea salt (low saline water) and 35 ppt sea salt (high saline water); B. Dissolved ozone concentration measured in tanks with LIG electrodes applied at 3 V in 0.5 ppt NaCl (low saline water) and 35 ppt NaCl (high saline water); C. Dissolved ozone concentration measured in tanks with LIG electrodes applied at 3 V in 3 ppt sea salt (moderately saline water) and 35 ppt sea salt (high saline water). The median value of three biological replicates is shown, and bars represent the 95% CI ($n=3$).

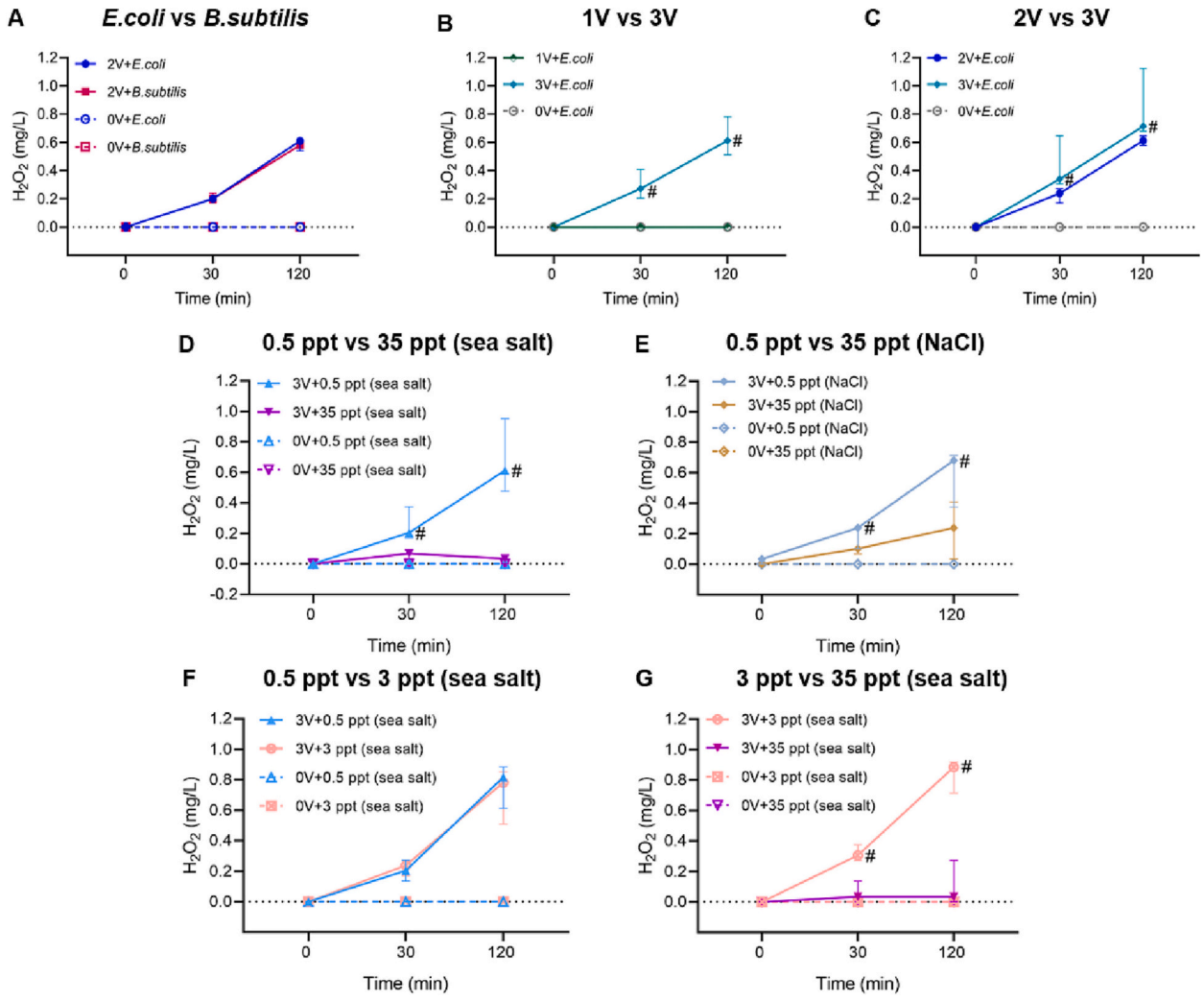

Fig. 6. Hydrogen peroxide (H_2O_2) concentration in seawater during EC treatment. A: Hydrogen peroxide concentration in slightly saline water (2.92 ppt NaCl) spiked with *E. coli* and *B. subtilis* after treatment with 2 V; B: Hydrogen peroxide concentration at 1 V and 3 V in slightly saline water (2.92 ppt NaCl); C: Hydrogen peroxide concentration at 2 V and 3 V in slightly saline water (2.92 ppt NaCl); D: Hydrogen peroxide concentration in 0.5 ppt sea salt (low saline water) and 35 ppt sea salt (high saline water) at 3 V; E: Hydrogen peroxide concentration in 0.5 ppt NaCl (low saline water) and 35 ppt NaCl (high saline water) at 3 V; F: Hydrogen peroxide concentration in 0.5 ppt sea salt (low saline water) and 3 ppt sea salt (moderately saline water) at 3 V; G: Hydrogen peroxide concentration in 3 ppt sea salt (moderately saline water) and 35 ppt sea salt (high saline water) at 3 V. The median value of three biological replicates is shown, and bars represent the 95% CI ($n=3$).

of hydrogen peroxide was also observed in low saline water (i.e., 0.5 ppt sea salt and 0.5 ppt NaCl) and moderately saline water (i.e., 3 ppt sea salt) compared to seawater (i.e., 35 ppt sea salt and 35 ppt NaCl) at 30 min ($p = 0.0463$) and 120 min ($p = 0.0369$) (Fig. 6D, E, and G). However, there was no significant difference in the concentration of hydrogen peroxide between the low and moderate salinity water groups (Fig. 5F). In the fish study, hydrogen peroxide was detected on day 4, day 7, and day 8, but its concentration was always below 0.1 mg/L.

4. Discussion

Our study confirms the rapid antibacterial performance of LIG electrodes for electrochemical disinfection (Singh et al., 2017; Singh et al., 2018), and expands on previous research to evaluate the disinfection effect of this material at different water salinities and on the health of fish. We found that LIG could not inactivate bacteria at very low salinity (i.e., 0.5 ppt); however, once the salinity was higher than 2.92 ppt, *E. coli* was eliminated within 120 min with as low as 2 V of electricity. Although the LIG electrodes in our study were effective for bacterial inactivation, the disinfection property of this material was voltage-dependent (Fig. 1B and C). Below 2 V, the disinfection property of LIG was not observed. This voltage-dependent inactivation was consistent with other studies using different electrode materials (Huang et al., 2016; Liao et al., 2017; Ni et al., 2020).

The LIG electrodes were effective at inactivating both Gram-negative (*E. coli*) and Gram-positive (*B. subtilis*) bacteria (Fig. 1A). However, Gram-negative bacteria may be more vulnerable to this technology than Gram-positive bacteria. Although not statistically significant with our sample size, we observed a trend suggesting a faster decline in *E. coli* over time than *B. subtilis*, with complete removal of *E. coli* after a 120 min treatment (Fig. 1A). The difference in the disinfection efficacy of the LIG treatment between Gram-negative and Gram-positive bacteria is consistent with previous findings (Ni et al., 2020; Xie et al., 2020). The finding may be attributed to the difference in the cell wall compositions of these two types of bacteria. The cell walls of Gram-negative bacteria are composed of phospholipid bilayers that can be readily hydrolyzed by hydroxide ions, which can be found around the cathode during electrolysis (Wang et al., 2020a). In contrast, the cell walls of Gram-positive bacteria are composed of a thick peptidoglycan layer, which may provide more protection (Zupanc et al., 2019).

The sterilization effect of electrochemical disinfection by the LIG electrodes was more effective and faster in higher salinity water. The mechanism of electrochemical disinfection is thought to be through the generation of oxidants such as reactive oxygen species (ROS) and reactive chlorine species (RCS) (Moreno-Andrés et al., 2018). Thus, it was expected that higher salinity water containing higher chloride ions would produce higher RCS, which may contribute to better antibacterial efficiency with increasing salinity. Several RCS have been detected and ascribed to the antimicrobial activity during electrochemical disinfection where titanium mesh, conductive diamond, and boron-doped diamond were used as electrode materials (Gil et al., 2019; Hsu et al., 2017; Lacasa et al., 2013). Despite the excellent disinfection properties of LIG electrodes in salt water in our study, we could not detect chlorine in any of our tanks during the electrochemical treatments. A possible reason for the lack of detectable chlorine was the limitation of the chlorine test strips used in our study. It is also possible that the RCSs were localized within LIG's highly porous foam-like structure (Singh et al., 2017) rather than distributed in the water, which aligns with a report on carbon nanotube electrodes with similar porous structure (Wang et al., 2020b). Our lack of chlorine detection was consistent with the findings from Norra et al. (2022) who used graphene sponge as electrode material to treat water containing 20 mM NaCl.

Other oxidants, particularly ozone and hydrogen peroxide, have also be generated during electrochemical treatment using other electrodes, such as boron-doped diamond electrodes (Liu et al., 2021), tin oxide electrodes (Wang et al., 2005), and aluminum granules electrodes (Jodpimai et al., 2015), and may be responsible for bacterial inactivation (Moreno-Andrés et al., 2018). In this study, dissolved ozone was only detected in high saline water (35 ppt sea salt, 35 ppt NaCl, or natural seawater) and increased with treatment time, reaching its highest level of 0.16 mg/L at 120 min in one of our tanks with natural seawater (Fig. 5). Although this level of ozone is low for the typical water disinfection threshold (i.e., >1.5 mg/L)) (Ding et al., 2019; Loeb et al., 2012), it could have a deleterious effect on bacteria if exposure time was sufficient.

We also detected hydrogen peroxide; however, the concentration of this oxidant was much lower than the threshold associated with disinfection (Singh et al., 2017). In addition, the concentration of hydrogen peroxide did not vary much between low and moderate salinity water, and was much lower in the high saline water (Fig. 6), which is contrary to what we would expect given the disinfection trend with salinity. However, the lack of hydrogen peroxide in high-salinity water may be explained by the chemical reaction of hydrogen peroxide with the RCS generated during electrochemical treatment (Steter et al., 2016).

Excessive chlorine, ozone, and hydrogen peroxide can be deleterious to some aquatic animals (Kolawole and Iyiola, 2021; Stiller et al., 2020; Vera and Migaud, 2016). In our study, there was non-detectable chlorine, and the ozone and hydrogen peroxide concentration were below the lethal concentration (LC50) for many aquatic species (Pumkaew et al., 2021; Vera and Migaud, 2016), suggesting this technology may be safe. Further evidence for the safety of this technology was that other water quality parameters (i.e., pH, DO, and temperature) were relatively stable (Fig. 4 and supplementary fig. 3) and within the normal range for many aquatic species (Abdel-Tawwab et al., 2019; Mousset et al., 2020; Wang et al., 2019). The LIG material has been demonstrated to be non-toxic to zebrafish, even though high concentrations of LIG powder were used (d'Amora et al., 2020). The fish study we conducted on Japanese medaka further supported the safety of the LIG technology. No mortality or abnormal behaviors were observed, nor were there any significant histological changes in fish gills after the electrochemical disinfection using LIG electrodes with 3 V of electricity. Other researchers have also reported safety usage of electrochemical disinfection in aquatic animals with electrodes of unknown material despite generating relatively high concentrations of chlorine and ozone during the electrochemical disinfection process (Jorquera et al., 2002; Katayose et al., 2007; Ye et al., 2017). Our study using LIG technology may circumvent this potential toxicity problem.

Besides safety, another concern that needs to be addressed before LIG technology is commercialized is the stability and durability of the product. Huang et al.(Huang et al., 2021) reported that the antiviral stability of LIG materials was above 60% after being reused three times. We also found a slight decline in the killing potential of the LIG electrodes after 4 days in our study. Defining the duration of efficacy of this new material and finding ways to improve the longevity of the product will be the key to its commercialization. Future research on this topic is underway (Gu et al., 2021).

Another consideration for the commercialization of LIG electrochemical disinfection is the cost of using the product. At this stage, it is difficult to estimate the cost of using this material on a commercial scale as it is still only laboratory tested. However, if we apply the energy cost suggested by Ni et al. (2020), the energy consumption of our lab-scale system would be estimated at ~0.005–0.016 W·h /m^3 per log removal of *E.coli*. In comparison, this was less than Ni et al. (2020) estimated for their small-scale disinfection system using graphitized carbon filter electrodes (estimated at 5.7–34.2 W·h /m^3 per log removal of *E.coli)*. Given this, our LIG material may be cost-effective; however, before a cost-benefit analysis is done, the system should be scaled up to represent the size of a commercial operation, as the efficiency would likely differ. Also, as technology improves, the cost should decline.

In conclusion, our findings indicate that electrochemical disinfection using the LIG technology may help safely reduce bacteria in aquatic saltwater systems. The low voltage and short disinfection time required

to disinfect water in our study are promising and warrant further investigation to determine if it can be scaled up to accommodate saltwater aquaculture systems. However, more research is required to thoroughly understand how LIG works to inactivate bacteria, the effective duration of this material, and how it can be scaled to a commercial setting. Further, this technology should also be evaluated on specific fish pathogens, including parasites and viruses. Our initial findings highlight that electrochemical treatments using LIG electrodes are a promising potential alternative to current disinfection methods used to control bacteria in seawater systems.

Submission declaration and verification

The work described has not been published previously, and it is not under consideration for publication elsewhere. This publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or any other language, including electronically without the written consent of the copyright-holder.

Author contributions

Ju Zhang, Ruquan Ye, and Sophie St-Hilaire contributed to the idea of the study. Ng Pok Him and Libei Huang assisted with experimental set-up and materials preparation. Ladan Jahangiri, Liqing Huang, Brett MacKinnon, Yin Yang, and Qianjun Huang were involved in data acquisition and manuscript revising. Andrew Ferguson helped with the histology. Omid Nekouei, Yefeng Yang, and Ana Rita Marques contributed to the statistical analysis.

Author statement

Details of each author with their contribution in this paper:

Name of the author and e-mail ID	Types of contribution
Ju Zhang: zhang.ju@my.cityu.edu.hk	conducted most of the studies and wrote the manuscript
Liqing Huang: liqhuang@cityu.edu.hk	Assisted with data acquisition and manuscript revising
Pok Him Ng: pokhimng@cityu.edu.hk	Assisted with experimental set-up, data acquisition and manuscript revising
Ladan JAHANGIRI: ljahangir2-c@my.cityu.edu.hk	Assisted with data acquisition and manuscript revising
Qianjun Huang: qjhuang2-c@my.cityu.edu.hk	Assisted with data acquisition and manuscript revising
Libei Huang: libehuang2-c@my.cityu.edu.hk	Assisted with materials preparation
Brett MacKinnon: brett.mackinnon@cityu.edu.hk	Assisted with data acquisition and manuscript revising
Ying Yang: cmu_yy@163.com	Assisted with data acquisition and manuscript revising
Omid Nekouei: omid.nekouei@cityu.edu.hk	Assisted with statistical analysis
Yefeng Yang: yefeyang@cityu.edu.hk	Assisted with statistical analysis
Andrew Ferguson:Andrew.ferguson@cityu.edu.hk	Assisted with data acquisition and manuscript revising
Ana Rita Marques: apinheir@cityu.edu.hk	Assisted with statistical analysis
Ruquan Ye: ruquanye@cityu.edu.hk	Assisted with the conception of the study
Sophie St-Hilaire: ssthilai@cityu.edu.hk	Assisted with the conception of the study and manuscript revisions

City University of Hong Kong.

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Declaration of Competing Interest

The authors declare no competing interests.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2023.739479.

References

Abdel-Tawwab, M., Monier, M.N., Hoseinifar, S.H., Faggio, C., 2019. Fish response to hypoxia stress: growth, physiological, and immunological biomarkers. Fish Physiol. Biochem. 45, 997–1013. https://doi.org/10.1007/s10695-019-00614-9.

Ali, I., Alharbi, O.M., Tkachev, A., Galunin, E., Burakov, A., Grachev, V.A., 2018. Water treatment by new-generation graphene materials: hope for bright future. Environ. Sci. Pollut. Res. 25, 7315–7329. https://doi.org/10.1007/s11356-018-1315-9.

Azhdari, R., Mousavi, S.M., Hashemi, S.A., Bahrani, S., Ramakrishna, S., 2019. Decorated graphene with aluminum fumarate metal organic framework as a superior non-toxic agent for efficient removal of Congo red dye from wastewater. J. Environ. Chem. Eng. 7, 103437 https://doi.org/10.1016/j.jece.2019.103437.

Barbhuiya, N.H., Kumar, A., Singh, S.P., 2021. A journey of laser-induced graphene in water treatment. Transact. Indian Nat. Acad. Eng. 6, 159–171. https://doi.org/10.1007/s41403-021-00205-2.

Ben-Asher, R., Lahav, O., 2019. Minimization of THM formation in seawater-fed recirculating aquaculture systems operated with electrochemical NH4+ removal. Aquaculture 502, 162–175. https://doi.org/10.1016/j.aquaculture.2018.12.025.

Bo, J., Cai, L., Xu, J.-H., Wang, K.-J., Au, D.W., 2011. The marine medaka Oryzias melastigma–a potential marine fish model for innate immune study. Mar. Pollut. Bull. 63, 267–276. https://doi.org/10.1016/j.marpolbul.2011.05.014.

Bruguera-Casamada, C., Sires, I., Brillas, E., Araujo, R.M., 2017. Effect of electrogenerated hydroxyl radicals, active chlorine and organic matter on the electrochemical inactivation of Pseudomonas aeruginosa using BDD and dimensionally stable anodes. Sep. Purif. Technol. 178, 224–231. https://doi.org/10.1016/j.seppur.2017.01.042.

Chuah, L.-O., Effarizah, M., Goni, A.M., Rusul, G., 2016. Antibiotic application and emergence of multiple antibiotic resistance (MAR) in global catfish aquaculture. Curr. Environ. Health Rep. 3, 118–127. https://doi.org/10.1007/s40572-016-0091-2.

Costello, C., Cao, L., Gelcich, S., Cisneros-Mata, M.Á., Free, C.M., Froehlich, H.E., Golden, C.D., Ishimura, G., Maier, J., Macadam-Somer, I., 2020. The future of food from the sea. Nature 588, 95–100. https://doi.org/10.1038/s41586-020-2616-y.

d'Amora, M., Lamberti, A., Fontana, M., Giordani, S., 2020. Toxicity assessment of laser-induced graphene by zebrafish during development. J. Phys. Mater. 3, 034008 https://doi.org/10.1088/2515-7639/ab9522.

Dbira, S., Bensalah, N., Ahmad, M.I., Bedoui, A., 2019. Electrochemical oxidation/disinfection of urine wastewaters with different anode materials. Materials 12, 1254. https://doi.org/10.3390/ma12081254.

Demirel, C.S.U., Birben, N.C., Bekbolet, M., 2018. A comprehensive review on the use of second generation TiO_2 photocatalysts: microorganism inactivation. Chemosphere 211, 420–448. https://doi.org/10.1016/j.chemosphere.2018.07.121.

Ding, W., Jin, W., Cao, S., Zhou, X., Wang, C., Jiang, Q., Huang, H., Tu, R., Han, S.F., Wang, Q., 2019. Ozone disinfection of chlorine-resistant bacteria in drinking water. Water Res. 160, 339–349. https://doi.org/10.1016/j.watres.2019.05.014.

Edwards, P., Zhang, W., Belton, B., Little, D.C., 2019. Misunderstandings, myths and mantras in aquaculture: its contribution to world food supplies has been systematically over reported. Mar. Policy 106, 103547. https://doi.org/10.1016/j.marpol.2019.103547.

Fan, M., Gong, L., Sun, J., Wang, D., Bi, F., Gong, Z., 2018. Killing two birds with one stone: coating Ag NPs embedded filter paper with chitosan for better and durable point-of-use water disinfection. ACS Appl. Mater. Interfaces 10, 38239–38245. https://doi.org/10.1021/acsami.8b13985.

Gil, M.I., López-Gálvez, F., Andújar, S., Moreno, M., Allende, A., 2019. Disinfection by-products generated by sodium hypochlorite and electrochemical disinfection in

different process wash water and fresh-cut products and their reduction by activated carbon. Food Control 100, 46–52. https://doi.org/10.1016/j.foodcont.2018.12.050.
Grob, C., Pollet, B.G., 2016. Regrowth in ship's ballast water tanks: think again! Mar. Pollut. Bull. 109, 46–48. https://doi.org/10.1016/j.marpolbul.2016.04.061.
Gu, M., Huang, L., Wang, Z., Guo, W., Cheng, L., Yuan, Y., Zhou, Z., Hu, L., Chen, S., Shen, C., et al., 2021. Molecular engineering of laser-induced graphene for potential-driven broad-Spectrum antimicrobial and antiviral applications. Small 17, 2102841. https://doi.org/10.1002/smll.202102841.
Guilherme, S., Crespo, R., Azevedo, D., Marques, A., Santos, M., Serradeiro, R., Pacheco, M., 2019. DNA and chromosomal damage in Senegalese sole (*Solea senegalensis*) as side effects of ozone-based water treatment-contribution to optimization of fish-farming practices. Comp. Biochem. Physiol. Part C: Toxicol. Pharmacol. 219, 68–76. https://doi.org/10.1016/j.cbpc.2019.02.001.
Hsu, G.-S.W., Lu, Y.-F., Hsu, S.-Y., 2017. Effects of electrolysis time and electric potential on chlorine generation of electrolyzed deep ocean water. J. Food Drug Anal. 25, 759–765. https://doi.org/10.1016/j.jfda.2016.07.001.
Hu, J., Chu, W., Sui, M., Xu, B., Gao, N., Ding, S., 2018. Comparison of drinking water treatment processes combinations for the minimization of subsequent disinfection by-products formation during chlorination and chloramination. Chem. Eng. J. 335, 352–361. https://doi.org/10.1016/j.cej.2017.10.144.
Huang, X., Qu, Y., Cid, C.A., Finke, C., Hoffmann, M.R., Lim, K., Jiang, S.C., 2016. Electrochemical disinfection of toilet wastewater using wastewater electrolysis cell. Water Res. 92, 164–172. https://doi.org/10.1016/j.watres.2016.01.040.
Huang, L., Ling, L., Su, J., Song, Y., Wang, Z., Tang, B.Z., Westerhoff, P., Ye, R., 2020. Laser-engineered graphene on wood enables efficient antibacterial, anti-salt-fouling, and lipophilic-matter-rejection solar evaporation. ACS Appl. Mater. Interfaces 12, 51864–51872. https://doi.org/10.1021/acsami.0c16596.
Huang, L., Gu, M., Wang, Z., Tang, T.W., Zhu, Z., Yuan, Y., Wang, D., Shen, C., Tang, B. Z., Ye, R., 2021. Highly efficient and rapid inactivation of coronavirus on non-metal hydrophobic laser-induced graphene in mild conditions. Adv. Funct. Mater. 31, 2101195. https://doi.org/10.1002/adfm.202101195.
Ikehata, K., 2019. Recent research on ozonation by-products in water and wastewater treatment: formation, control, mitigation, and other relevant topics. Water Wastewater Treatment Technol. 117–144. https://doi.org/10.1007/978-981-13-3259-3_7.
Isidro, J., Brackemeyer, D., Sáez, C., Llanos, J., Lobato, J., Cañizares, P., Matthée, T., Rodrigo, M., 2020. Electro-disinfection with BDD-electrodes featuring PEM technology. Sep. Purif. Technol. 248, 117081 https://doi.org/10.1016/j.seppur.2020.117081.
Jodpimai, S., Boonduang, S., Limsuwan, P., 2015. Dielectric barrier discharge ozone generator using aluminum granules electrodes. J. Electrost. 74, 108–114. https://doi.org/10.1016/j.elstat.2014.12.003.
Jorquera, M.A., Valencia, G., Eguchi, M., Katayose, M., Riquelme, C., 2002. Disinfection of seawater for hatchery aquaculture systems using electrolytic water treatment. Aquaculture 207, 213–224. https://doi.org/10.1016/S0044-8486(01)00766-9.
Katayose, M., Yoshida, K., Achiwa, N., Eguchi, M., 2007. Safety of electrolyzed seawater for use in aquaculture. Aquaculture 264, 119–129.
Kolawole, A.S., Iyiola, A.O., 2021. Acute toxicity of calcium hypochlorite (65% chlorine disinfectant) on the liver, brain, and gill of *Clarias gariepinus* fingerlings (Burchell, 1822). Proceed. Nigerian Acad. Sci. 13 https://doi.org/10.5423/pngas.v13i2.
Lacasa, E., Tsolaki, E., Sbokou, Z., Rodrigo, M.A., Mantzavinos, D., Diamadopoulos, E., 2013. Electrochemical disinfection of simulated ballast water on conductive diamond electrodes. Chem. Eng. J. 223, 516–523. https://doi.org/10.1016/j.cej.2013.03.003.
Lacasa, E., Cotillas, S., Saez, C., Lobato, J., Cañizares, P., Rodrigo, M., 2019. Environmental applications of electrochemical technology. What is needed to enable full-scale applications? Curr. Opin. Electrochem. 16, 149–156. https://doi.org/10.1016/j.coelec.2019.07.002.
Li, M.-F., Liu, Y.-G., Zeng, G.-M., Liu, N., Liu, S.-B., 2019. Graphene and graphene-based nanocomposites used for antibiotics removal in water treatment: a review. Chemosphere 226, 360–380. https://doi.org/10.1016/j.chemosphere.2019.03.117.
Li, S., Tao, Y., Li, D., Wen, G., Zhou, J., Manickam, S., Han, Y., Chai, W.S., 2021. Fermentation of blueberry juices using autochthonous lactic acid bacteria isolated from fruit environment: Fermentation characteristics and evolution of phenolic profiles. Chemosphere 276, 130090. https://doi.org/10.1016/j.chemosphere.2021.130090.
Liao, X., Xuan, X., Li, J., Suo, Y., Liu, D., Ye, X., Chen, S., Ding, T., 2017. Bactericidal action of slightly acidic electrolyzed water against *Escherichia coli* and *Staphylococcus aureus* via multiple cell targets. Food Control 79, 380–385. https://doi.org/10.1016/j.foodcont.2017.03.050.
Liu, S.-S., Qu, H.-M., Yang, D., Hu, H., Liu, W.-L., Qiu, Z.-G., Hou, A.-M., Guo, J., Li, J.-W., Shen, Z.-Q., 2018. Chlorine disinfection increases both intracellular and extracellular antibiotic resistance genes in a full-scale wastewater treatment plant. Water Res. 136, 131–136. https://doi.org/10.1016/j.watres.2018.02.036.
Liu, F., Deng, Z., Miao, D., Chen, W., Wang, Y., Zhou, K., Ma, L., Wei, Q., 2021. A highly stable microporous boron-doped diamond electrode etched by oxygen plasma for enhanced electrochemical ozone generation. J. Environ. Chem. Eng. 9, 106369 https://doi.org/10.1016/j.jece.2021.106369.
Loeb, B.L., Thompson, C.M., Drago, J., Takahara, H., Baig, S., 2012. Worldwide ozone capacity for treatment of drinking water and wastewater: a review. Ozone Sci. Eng. 34, 64–77. https://doi.org/10.1080/01919512.2012.640251.
Martínez-Huitle, C.A., Brillas, E., 2021. A critical review over the electrochemical disinfection of bacteria in synthetic and real wastewaters using a boron-doped diamond anode. Curr. Opinion Solid State Mater. Sci. 25, 100926 https://doi.org/10.1016/j.cossms.2021.100926.
Mecha, A.C., Onyango, M.S., Ochieng, A., Momba, M.N., 2017. Evaluation of synergy and bacterial regrowth in photocatalytic ozonation disinfection of municipal wastewater. Sci. Total Environ. 601, 626–635. https://doi.org/10.1016/j.scitotenv.2017.05.204.
Moreno-Andrés, J., Ambauen, N., Vadstein, O., Hallé, C., Acevedo-Merino, A., Nebot, E., Meyn, T., 2018. Inactivation of marine heterotrophic bacteria in ballast water by an electrochemical advanced oxidation process. Water Res. 140, 377–386. https://doi.org/10.1016/j.watres.2018.04.061.
Mousset, E., Quackenbush, L., Schondek, C., Gerardin-Vergne, A., Pontvianne, S., Kmiotek, S., Pons, M.-N., 2020. Effect of homogeneous Fenton combined with electron transfer on the fate of inorganic chlorinated species in synthetic and reclaimed municipal wastewater. Electrochim. Acta 334, 135608. https://doi.org/10.1016/j.electacta.2019.135608.
Ni, X.-Y., Liu, H., Xin, L., Xu, Z.-B., Wang, Y.-H., Peng, L., Chen, Z., Wu, Y.-H., Hu, H.-Y., 2020. Disinfection performance and mechanism of the carbon fiber-based flow-through electrode system (FES) towards Gram-negative and Gram-positive bacteria. Electrochim. Acta 341, 135993. https://doi.org/10.1016/j.electacta.2020.135993.
Norra, G.-F., Baptista-Pires, L., Lumbaque, E.C., Borrego, C.M., Radjenovic, J., 2022. Chlorine-free electrochemical disinfection using graphene sponge electrodes. Chem. Eng. J. 430, 132772 https://doi.org/10.1016/j.cej.2021.132772.
Periyasamy, S., Lin, X., Ganiyu, S.O., Kamaraj, S.-K., Thiam, A., Liu, D., 2022. Insight into BDD electrochemical oxidation of florfenicol in water: kinetics, reaction mechanism, and toxicity. Chemosphere 288, 132433. https://doi.org/10.1016/j.chemosphere.2021.132433.
Pumkaew, M., Taweephitakthai, T., Satanwat, P., Yocawibun, P., Chumtong, P., Pungrasmi, W., Powtongsook, S., 2021. Use of ozone for *Vibrio parahaemolyticus* inactivation alongside nitrification biofilter treatment in shrimp-rearing recirculating aquaculture system. J. Water Process Eng. 44, 102396 https://doi.org/10.1016/j.jwpe.2021.102396.
Reddy, C.V., Reddy, I.N., Reddy, K.R., Jaesool, S., Yoo, K., 2019. Template-free synthesis of tetragonal co-doped ZrO_2 nanoparticles for applications in electrochemical energy storage and water treatment. Electrochim. Acta 317, 416–426. https://doi.org/10.1016/j.electacta.2019.06.010.
Rivas, A.J., Lemos, M.L., Osorio, C.R., 2013. Photobacterium damselae subsp. damselae, a bacterium pathogenic for marine animals and humans. Front. Microbiol. *4*, 283. https://doi.org/10.3389/fmicb.2013.00283.
Schaefer, C.E., Andaya, C., Urtiaga, A., 2015. Assessment of disinfection and by-product formation during electrochemical treatment of surface water using a Ti/IrO_2 anode. Chem. Eng. J. 264, 411–416. https://doi.org/10.1016/j.cej.2014.11.082.
Serna-Galvis, E.A., Berrio-Perlaza, K.E., Torres-Palma, R.A., 2017. Electrochemical treatment of penicillin, cephalosporin, and fluoroquinolone antibiotics via active chlorine: evaluation of antimicrobial activity, toxicity, matrix, and their correlation with the degradation pathways. Environ. Sci. Pollut. Res. 24, 23771–23782. https://doi.org/10.1007/s11356-017-9985-2.
Singh, S.P., Li, Y., Be'er, A., Oren, Y., Tour, J.M., Arnusch, C.J., 2017. Laser-induced graphene layers and electrodes prevents microbial fouling and exerts antimicrobial action. ACS Appl. Mater. Interfaces 9, 18238–18247. https://doi.org/10.1021/acsami.7b04863.
Singh, S.P., Li, Y., Zhang, J., Tour, J.M., Arnusch, C.J., 2018. Sulfur-doped laser-induced porous graphene derived from polysulfone-class polymers and membranes. ACS Nano 12, 289–297. https://doi.org/10.1021/acsnano.7b06263.
Stentiford, G., Peeler, E., Tyler, C., Bickley, L., Holt, C., Bass, D., Turner, A., Baker-Austin, C., Ellis, T., Lowther, J., 2022. A seafood risk tool for assessing and mitigating chemical and pathogen hazards in the aquaculture supply chain. Nat. Food 3, 169–178. https://doi.org/10.1038/s43016-022-00465-3.
Steter, J.R., Brillas, E., Sirés, I., 2016. On the selection of the anode material for the electrochemical removal of methylparaben from different aqueous media. Electrochim. Acta 222, 1464–1474. https://doi.org/10.1016/j.electacta.2016.11.125.
Stiller, K.T., Kolarevic, J., Lazado, C.C., Gerwins, J., Good, C., Summerfelt, S.T., Mota, V. C., Espmark, Å.M., 2020. The effects of ozone on Atlantic salmon post-smolt in brackish water—establishing welfare indicators and thresholds. Int. J. Mol. Sci. 21, 5109. https://doi.org/10.3390/ijms21145109.
Vera, L., Migaud, H., 2016. Hydrogen peroxide treatment in Atlantic salmon induces stress and detoxification response in a daily manner. Chronobiol. Int. 33, 530–542. https://doi.org/10.3109/07420528.2015.1131164.
Wang, Y.-H., Cheng, S., Chan, K.-Y., Li, X.Y., 2005. Electrolytic generation of ozone on antimony-and nickel-doped tin oxide electrode. J. Electrochem. Soc. 152, D197. https://doi.org/10.1149/1.2041007.
Wang, S., Jiang, Z., Mousavi, S.E., 2019. Effect of seawater pH on selected blood biochemical parameters of juvenile turbot *Scophthalmus maximus* (Linnaeus, 1758). Indian J. Fish 66, 78–83. https://doi.org/10.21077/ijf.2019.66.4.92173-10.
Wang, S., Zhu, Y., Yang, Y., Li, J., Hoffmann, M.R., 2020a. Electrochemical cell lysis of gram-positive and gram-negative bacteria: DNA extraction from environmental water samples. Electrochim. Acta 338, 135864. https://doi.org/10.1016/j.electacta.2020.135864.
Wang, X., Sun, M., Zhao, Y., Wang, C., Ma, W., Wong, M.S., Elimelech, M., 2020b. In situ electrochemical generation of reactive chlorine species for efficient ultrafiltration membrane self-cleaning. Environ. Sci. Technol. 54, 6997–7007. https://doi.org/10.1021/acs.est.0c01590.
Xie, L., Shu, Y., Hu, Y., Cheng, J., Chen, Y., 2020. SWNTs-PAN/TPU/PANI composite electrospun nanofiber membrane for point-of-use efficient electrochemical disinfection: new strategy of CNT disinfection. Chemosphere 251, 126286. https://doi.org/10.1016/j.chemosphere.2020.126286.
Yang, Y., Pignatello, J.J., Ma, J., Mitch, W.A., 2016. Effect of matrix components on UV/H_2O_2 and UV/$S_2O_8^{2-}$ advanced oxidation processes for trace organic degradation in

reverse osmosis brines from municipal wastewater reuse facilities. Water Res. 89, 192–200. https://doi.org/10.1016/j.watres.2015.11.049.

Ye, Z., Wang, S., Gao, W., Li, H., Pei, L., Shen, M., Zhu, S., 2017. Synergistic effects of micro-electrolysis-photocatalysis on water treatment and fish performance in saline recirculating aquaculture system. Sci. Rep. 7, 1–12. https://doi.org/10.1038/srep45066.

Zhu, J., Cho, M., Li, Y., Cho, I., Suh, J.-H., Orbe, D.D., Jeong, Y., Ren, T.-L., Park, I., 2019. Biomimetic turbinate-like artificial nose for hydrogen detection based on 3D porous laser-induced graphene. ACS Appl. Mater. Interfaces 11, 24386–24394. https://doi.org/10.1021/acsami.9b04495.

Zupanc, M., Pandur, Ž., Perdih, T.S., Stopar, D., Petkovšek, M., Dular, M., 2019. Effects of cavitation on different microorganisms: the current understanding of the mechanisms taking place behind the phenomenon. A review and proposals for further research. Ultrason. Sonochem. 57, 147–165. https://doi.org/10.1016/j.ultsonch.2019.05.009.