Research Article

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Degradation of *Vibrio cholerae* from drinking water by the underwater capillary discharge

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Abstract: Underwater plasma discharge is considered a nontoxic and effectual purification approach to control waterborne bacterial pathogens. In the present study, *Vibrio cholerae* contaminated drinking water was sterilized by using underwater capillary discharge generation via high voltage, oxygen (O_2) injection, and hydrogen peroxide (H_2O_2) addition. The effects of oxidant species generated by plasma discharge on V. cholerae disinfection have been studied and reported. The electrical and optical analysis of capillary discharge revealed the generation of reactive oxygen species (OH_2, H_1) and (OH_2, H_2) from the water after (OH_2, H_2) injected and (OH_2, H_2) added plasma discharge was

achieved, and 100% inactivation of $V.\ cholera$ from drinking water was proven. The retardant effect of the initial $V.\ cholerae$ colonies with time variation was reported through optical density $(\mathrm{OD_{600\,nm}})$ measurements. The time course study of bactericidal activity of plasma treatment on $V.\ cholerae$, observed every 12 h up to 36 h, revealed a high retardant effect on $V.\ cholerae$ CFUs. Underwater capillary discharge is an efficient approach for the inactivation of $V.\ cholerae$ from drinking water.

Keywords: capillary discharge, *V. cholerae*, antibacterial activity, water-borne pathogens

1 Introduction

V. cholerae causes major health issues in more than 50 countries around the world. The presence of V. cholerae in water grows dynamically and in continuous recurring cycles [1,2]. The climate factors such as rainfall, floods, human exposure and sanitary conditions, population growth, and land development along river banks and near underwater reservoirs affect the V. cholerae concentrations in the environment, especially in drinking water [3,4]. Many common methods for regulating the abovementioned bacteria from drinking water have been used in the past [5–8]. Sterilization of waterborne pathogens such as V. cholera, by low-temperature arc plasma, has proved to be very effective in the last few decades [9]. In the past, many pioneer works and industrial device developments including DC-powered plasma jets, RFinduced devices, dielectric barrier discharges, based on plasma generation, in the context of antibacterial applications, for mitigating agrochemical and pesticide residue in foods and water, plasma medicines, and hygiene and in health sciences, were investigated and reported [10–13]. Underwater capillary discharge is a novel technique for water purification and has been proven an effective approach in the last few decades [14]. The occurrence of arc discharge in capillary tubes containing flowing water leads to the

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generation of highly reactive oxidant species (ROS), including OH radicals, reactive hydrogen (H), reactive oxygen (O), ozone (O_3) , hydrogen peroxide (H_2O_2) (due to the dissociation of water molecules within the water medium), and the generation of intensive shockwaves was proven to be very effective for inactivating bacteria from water [15]. Some of the species like OH radicals are very short-lived (10⁻⁸ s), but due to high redox potential they react efficiently and quickly for the treatment of contaminated water of large volume. while other oxidant species like ozone (O₃) and hydrogen peroxide (H₂O₂) remain active for a longer time in water and could act as a sterilization agent for a longer time [16]. The main reason for using oxygen as an injected gas is to generate a large amount of ROS, which is highly useful for water purification [17,18]. Other outcomes of the discharge include shockwaves intense electric fields and UV radiations that are also highly useful for water purification [19-21]. The intense electric field reacts with the cell membranes of bacteria, overcomes its tensile strength, and causes its rupture. The electric field accumulates electric charges in the discharge region, and the interaction of electric charges with the bacterial cell membrane causes cell stress and its rupturing, making it unable to reproduce [22-27]. Refs. [28-36] highlight different important studies on the material review and literature.

In the present research, underwater capillary discharge was performed to neutralize frequently occurring waterborne pathogens, *i.e.*, *Vibrio cholera*.

2 Materials and methods

2.1 Vibrio cholerae strains and growth conditions

V. cholerae strains (O1) belonging to two unique subclades, *i.e.*, PSC1 and PSC2, were obtained from the Microbiology and Public Health Lab, COMSATS University, Islamabad, Pakistan. These strains were revived on thiosulfate–citrate–bile salts–sucrose (TCBS) (Sigma-Aldrich, Ireland Ltd.) agar plates. For each set of experiments, overnight cultures (16–18 h) of *V. cholerae* strains were grown in Luria broth (Lenox Invitrogen 22700025) at 37°C.

2.2 Bacterial cell survival assay

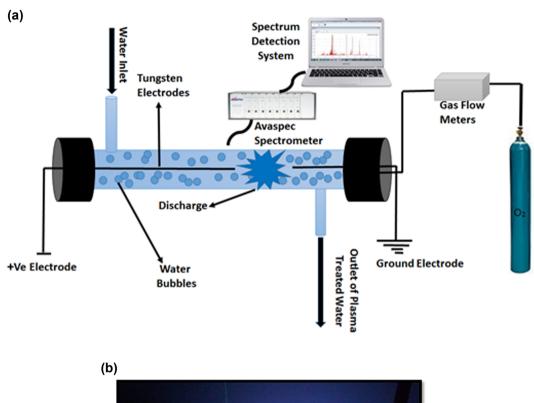
The effect of plasma treatment on bacterial growth and cell survival was determined under various experimental conditions. An overnight culture of *V. cholerae* strains

 $(\sim\!10^7\,\text{CFUs})$ was used to prepare the inoculum of bacteria suspension $(\sim\!10^3\,\text{CFU/mL})$ in 1 L of autoclaved distilled water. These suspensions were used to prepare different groups of experimental design $(0.35\,\text{mL/L}\,H_2O_2$ and 0 mL/L $H_2O_2)$ and were then exposed to plasma at a flow rate of 0.1 L/min through discharge containing a capillary tube. After plasma treatment of the water that contained bacteria, $100\,\mu\text{L}$ of the water suspension was plated on TCBS agar plates using a spreader, and the plates were incubated at 37°C for $24\,\text{h}$.

3 Experimental setup

The flowing water plasma discharge was created in a quartz capillary tube (length, 12 cm; outer diameter, 4 mm; and inner diameter, 2 mm with a thickness of 2 mm) specifically designed with a water inlet and outlet segments, along with gas injection facility for creating bubbles. A high-frequency (758 kHz) alternating current (AC) plasma generator CTP-2000S was used for applying a voltage across pin-pin configured tungsten electrodes, each of diameter 1 mm. Figure 1(a) represents the schematic diagram of the experimental setup, and Figure 1(b) represents the visual view of the capillary discharge.

The inter-electrode gap where the plasma was generated was 5 mm and the water flow rate was kept at 0.1 L/min. A mass flow controller (TELEDYNE-500P) was used for controlling the flow of oxygen (100-800 sccm, with 100 sccm trail). The emission spectrum of capillary discharge was recorded on an Avantes Avaspec-NIR256 miniature fiber-optic spectrometer. The spectrum shows the concentration of highly oxidant reactive species generated by underwater capillary discharge. The V. cholerae containing water was treated by plasma discharge under specific experimental conditions including various oxygen injected rates and with and without hydrogen peroxide (H₂O₂) addition. A standard amount of H_2O_2 (0.35 mL/L) was added for generating immense consolidation of the oxidant species. One liter of water reservoir containing 10⁵ CFUs of V. cholerae was introduced through the discharge tube along with oxygen injection by using an injecting syringe for spawning oxygen bubbles in flowing water. A digital oscilloscope (GWINS-TEK) having high voltage (peak-peak, 39 kV, DC/AC, rms, 27 kV) and large current probes (1 mA to 40 A with an effective frequency bandwidth range up to 50 MHz) and data storage facility was used for recording volt-ampere characteristics of flowing water capillary discharge. Sterilized water was tested directly after plasma treatment and examined every 12 h, up to 36 h to detect the growth of V. cholerae in water.



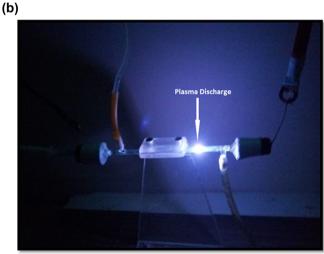


Figure 1: (a) Schematic diagram of the experimental setup. (b) A visual view of capillary discharge.

4 Results and discussion

The electrical, optical, and biological diagnostics techniques were adopted to study and report the inactivation of water-borne pathogens, *V. cholerae*, from drinking water.

4.1 Electrical results

The volt–ampere characteristics of capillary discharge were measured by a digital oscilloscope (GDS-3504). A high-voltage probe (TT-HCP 2739) having a 1,000 attenuation factor, a high input impedance (900 M Ω), and a large

current probe (GCP-530) with a current range of 1 mA to 40 A and an effective frequency bandwidth up to 50 MHz were used for determining the breakdown voltages, discharge currents, and power and energy of discharge pulses at various experimental schemes. Typical *I–V* curves are presented in Figure 2, after discharge occurrence. Since the dielectric strength of the tap water used in this experiment is quite high, enormous power is required to create a breakdown in the flowing water. In order to overcome this challenge, gas bubbles were introduced inside the capillary tube that induced elongated gas channels and bubbles inside the capillary tube and caused a reduction in the required breakdown voltage. In this experiment, oxygen

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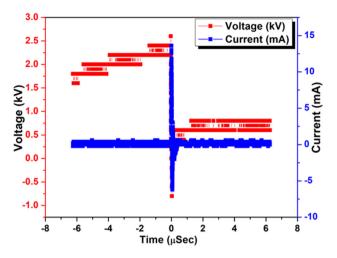


Figure 2: Typical volt-ampere characteristics curve.

was chosen as the preferred gas for creating flowing water discharge because oxygen has the ability to generate a high concentration of oxidant species (OH·, H_{α} , H_{β} , O_3 , H_2O_2). At various higher oxygen injection rates (100–800 sccm), the energy and power of discharge pulses have shown an incremental increase. Figure 3(a and b) represents the variations in energy and power of discharge pulses with and without H_2O_2 addition and for different oxygen injection rates.

The increase in oxygen injection rates caused an increase in the size and elongation of gas bubbles and gas channels inside the capillary tube, which drastically enhanced the power and energy of discharge pulses by limiting the required breakdown voltage. Such an increase in the power and energy of discharge pulses not only enhanced the yield rate of oxidant species by dissociating water molecules but also played a critical role in

disinfecting V. cholerae. The results showed that the addition of hydrogen peroxide (H_2O_2) does not cause a remarkable increase in water conductivity; therefore, electrical characteristics were not influenced by the addition of hydrogen peroxide (H_2O_2) . But the addition of hydrogen peroxide (H_2O_2) along with the occurrence of discharge caused a remarkable increase in the concentration of the oxidant species (Figure 5).

4.2 Optical diagnostics

An optical emission spectrum was used to examine the ROS generated by plasma discharge in capillary tubes [37]. The short reactive species such as OH radicals were present. Optical emission spectrometry has been frequently studied ($10^{-8}\,\mathrm{s}$) as well as long-lived reactive species (H_2O_2 and O_3) along with reactive hydrogen ($H\alpha$, H_β) and atomic oxygen (O_2). Different types of spectral lines represent the concentration of specific oxidant species in different spectral regions (visible, UV, and IR).

An Ava-Spec spectrometer (AVS-RACKMOUNT-USH2) with a resolution of 0.06–13 nm and a wavelength of 200–900 nm was used to determine the concentration of ROS produced by capillary discharge. Figure 4 shows the emission spectrum of effective reactive oxidizing species under various experiential conditions, showing the dominant spectral lines produced by the highly reactive oxidizing species resulting from the dissociation of water molecules through electrical energy. In the IR region, most descriptive lines were expected due to atomic oxygen, and the dominant peaks of hydrogen lines (Balmer $\alpha = 656$ nm and Balmer $\beta = 484$ nm) are present.

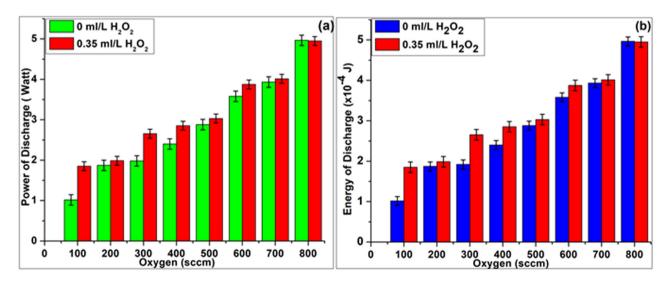


Figure 3: (a) Power and (b) energy of discharge pulses under various experimental conditions.

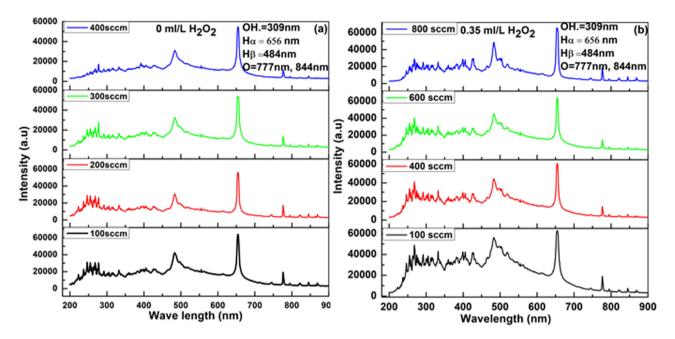


Figure 4: (Color online) A typical optical emission spectrum for the identification of ROS for (a) O_2 injection and 0 mL/L H_2O_2 addition; (b) O_2 injection and 0.35 mL/L H_2O_2 addition in water.

Powerful OH radical peaks have been examined in the UV spectrum at 309 nm. No distinctive lines are examined in the spectral line due to metal ablation from electrodes. Other dominant oxidizing species like H_2O_2 and

 $\rm O_3$ can be generated due to possible chemical reactions that take place when discharged into water [38,39]. The addition of $\rm H_2O_2$ together with oxygen-injected discharge increased the oxidant species concentration. Each oxidant

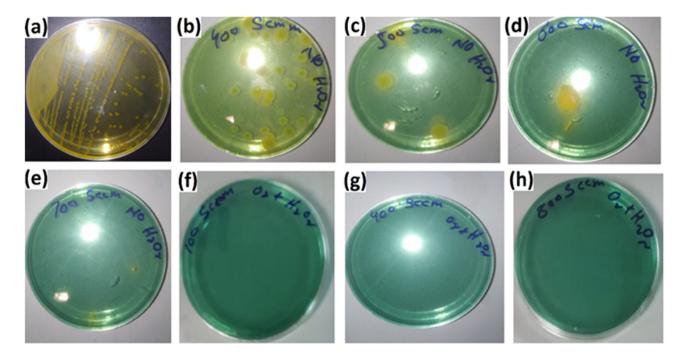


Figure 5: (Color online) Typical images of *Vibrio cholera* CFUs: (a) pure *V. cholerae* strain. CFUs obtained after (b) 400 sccm oxygen injection and 0 mL/L H_2O_2 addition, (c) 500 sccm oxygen injection and 0 mL/L H_2O_2 addition, (d) 600 sccm oxygen injection and 0 mL/L H_2O_2 addition, (e) 700 sccm oxygen injection and 0 mL/L H_2O_2 addition, (f) 100 sccm oxygen injection and 0.35 mL/L H_2O_2 addition, (g) 400 sccm oxygen injection and 0.35 mL/L H_2O_2 addition, and (h) 800 sccm oxygen injection and 0.35 mL/L H_2O_2 addition.

species (OH, H_2O_2 , O_3 , $H\alpha$, H_β , and O) have the potential for redox and water sterilization, but their individual effects are not very effective compared to their cumulative effects influencing V. cholera sterilization [40]. The concentration of these oxidant species was weak in the absence of the addition of hydrogen peroxide (H_2O_2); however, in the case of added hydrogen peroxide (H_2O_2) together with oxygen (O_2) injected in a capillary discharge, a remarkable increase was observed. More increased concentration was observed at higher oxygen injection rates. Although shortlived, OH radicals are effectively massacred by V. cholerae due to high redox potential (270 V). The discharge-generated, water dissolved H_2O_2 , and O_3 also have exceptional antibacterial properties, so V. cholerae disinfection was also effectively of concern.

4.3 Plasma discharge effect on *V. cholerae* inactivation and re-growth

After plasma treatment of bacteria containing water, the water was centrifuged (120 rpm) in a shaker and then 100 μ L of the water suspension was plated on TCBS agar plates using a spreader, and the plates were incubated at 37°C for 24 h. The CFUs on each plate were counted, and the CFUs/mL were calculated by using the following formula:

Colony forming unit (CFU/mL)
$$= \frac{\text{No. of colonies} \times \text{Dilution factor}}{\text{Volume of culture plated}}. \tag{1}$$

Untreated water suspension and inoculated water were used as positive and negative controls. Figure 5(a) represents the control V. cholerae strain before plasma treatment. Figure 5(b-h) represents typical results of plasma-treated V. cholerae containing water under different experimental conditions, with and without the addition of H_2O_2 . The results revealed that pure V. cholerae make a high concentration of colonies (10^7 CFUs), while after plasma treatment the colonies were reduced enormously as shown in images of TCBS plates. The addition of hydrogen peroxide (H_2O_2) and oxygen (O_2) injected in a discharge-created oxidant species (OH, H, and O), as represented in spectral results that played a vital role in sterilizing V. cholerae completely from drinking water.

The killing efficiency η (in %) of the plasma was calculated using the following formula and presented in Figure 6:

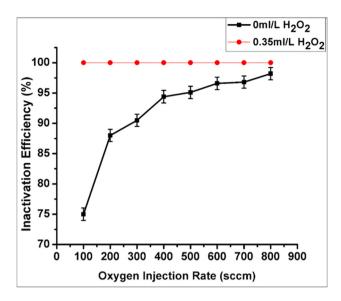


Figure 6: (Color online) Reliance of V. cholerae inactivation efficiency ' η ' on various oxygen injection rates and with the addition of H_2O_2 in underwater plasma discharges.

The logarithmic value of bactericidal colonies, which represents the kill rate, before and after the plasma treatment was calculated as

Kill rate =
$$\log \frac{N}{N_0}$$
, (3)

where N is the number of untreated colonies and N_0 is the number of colonies after plasma treatment. The kill rate is graphically illustrated in Figure 7.

Under optimum experimental conditions, the plasma-processed water samples were diagnosed after each 12 h interval, up to 36 h. Interestingly, the results demonstrated that with a higher oxygen injection rate and $\rm H_2O_2$ addition to water, the inactivation efficiency of V. cholerae increased remarkably. Figure 8 represents the retardant effect of plasma-treated water observed up to 36 h, in 12 h of timespace each. The V. cholerae survival percentage was calculated by the following relation:

% Survival of V. cholera
$$= \frac{\text{(OD of test group)} - \text{(OD of blank)}}{\text{(OD of control)} - \text{(OD of blank)}} \times 100.$$
(4)

The OD of the blank was 0.03345, the OD of pure strain was 0.91, and the OD of the control was 0.405; the OD of the test group varied for different plasma treatment

Killing efficiency (
$$\eta$$
) % = $\left(1 - \frac{\text{No. of CFU/mL in treated samples}}{\text{No. of CFU/mL in untreated control samples}}\right) \times 100.$ (2)

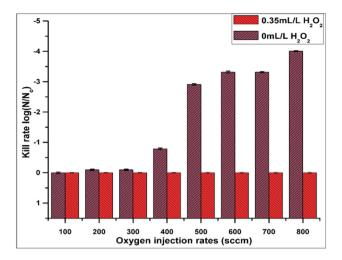


Figure 7: (Color online) Kill rate (the reduction in V. cholerae colonies) $vs \ O_2$ injection and H_2O_2 addition in water.

conditions. Figure 9 represents the kinetics and % viability of *V. cholerae* under different treatment conditions.

The O_2 -injected and H_2O_2 -added discharge resulted in higher anti-bacterial effects compared to the only O_2 -or H_2O_2 -injected discharge. The presence of electrical discharge creates highly intense shock waves and UV radiations, which not only destroy the DNA structure of bacteria but also cause a high dissociation rate of water molecules that generate an excess of oxidant species that are more amplified by O_2 injection and H_2O_2 addition. The presence of plasma between two water inserted electrodes causes thermal effects in water, which also plays an important role in V. cholerae disinfection. The plasmatreated water was observed after each 12 h by new agar

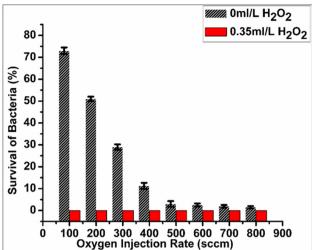


Figure 9: (Color online) Survival of V. cholerae in water after plasma treatment at various oxygen-injected rates with and without H_2O_2 addition in water.

plates that were preserved at -20 °C, and no increase in colonies was observed.

5 Conclusions

Underwater capillary discharge is proven to be an efficient and non-toxic method of water purification from V. *cholerae*. The addition of H_2O_2 in V. *cholerae* containing water and its treatment with O_2 -injected discharge at various O_2 injection rates (100–800 sccm) with a step of 100 sccm can generate large concentrations of highly

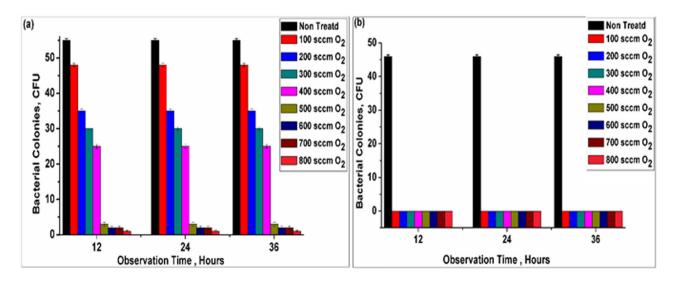


Figure 8: Retardant effect on the colony growth rate of plasma treated water observed after every 12 h up to 36 h (a) O_2 injected 0 mL/L H_2O_2 added treated samples (b) O_2 injected 0.35 mL/L H_2O_2 added treated samples.

oxidant reactive species (OH, H_2O_2 , O_3 , H_α , H_β , and O); moreover UV radiations, shock waves, electrical field, and thermal effects play a vital role in sterilizing drinking water from *V. cholerae*. Complete elimination of *V. cholerae* from drinking water can be achieved by O_2 injection and H_2O_2 addition by underwater capillary discharge. This research will provide a foundation for practical applications for the sterilization of large volumes of drinking water more effectively.

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