Chapter 26

Cold Plasma for Pathogenic Sterilization

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26.1 Introduction to Cold Plasma Sterilization Scheme

Pathogenic micro-organisms have been one of the oldest historical sources causing diseases among humans. Even in times of huge progress made by modern human race, conventional medicine often fails to counter swiftly during a pathogenic outbreak. SARS, Ebola and ongoing COVID-19 have all pressed for the need of urgent development of novel approaches for their eradication. In present days, conventional sterilization methods use low-temperature steam, ionizing radiation, H_2O_2 and hot air. Over the last decade, development in this technique focused on the following factors: low temperature operation, shorter operational cycles, environmentally safe, and price reduction. Cold plasma is also a useful mean satisfying all these needs. Presently, the waves of COVID-19 have triggered a worldwide search to find quick, effective solutions for sterilization. Atmospheric pressure cold plasma technologies have already been tested eradication of laboratory strain bacteria (*Aeromonas*) and its bacteriophage (virus of bacteria). Two different cold plasma devices are to be tested here for their sterilization capabilities.

1. Microwave Cold Plasma Jet (Device: A, Fig. 26.1a)
2. RF - Hollow Cathode Cold Plasma Device (Device B, Fig. 26.1b)

Rigorous optical emission spectroscopic (OES) studies of plasma were performed to understand the reason behind cold plasma disinfection. Results indicated that both devices release OH radicals by dissociation of H_2O_2 and / or H_2O (using dry mist through nebulizer) in the cold plasma which is preferable for the destruction of both *Aeromonas* bacteria and its phage virus (bacteriophage). It was seen that the larger area device has upper hand compared to the pencil like device as it would generate OH radicals even from atmospheric moisture. It is worthwhile to mention that bacteriophage is often touted in literature as more difficult to destroy when compared with coronavirus. Thus, that these devices might have the capability for destruction of COVID-19 and similar virus strains. They can be used for sterilization of COVID-19 wards of hospitals after sui testing, eliminating the necessity of using any traditional chemical sterilization process.

26.2 Efficacy of Cold Plasma Sterilization Process

In this section, it will be tried to devise experimental schemes to study the efficacy of plasma sterilization process with the help of two devices introduced in the previous section.

Device A

Experimental set up is shown schematically in Fig. 26.1a which includes the pen-like device A that operates at 2.45 GHz at low operating power (< 100 watts). The design consists of a stainless steel (SS) hollow outer cylinder with a solid electrode placed at its centre. Argon (Ar) gas is passed through a container of heated H_2O_2 to produce plasma here. Ar carries H_2O_2 vapor which dissociates inside plasma to produce biocidal hydroxyl and hydroproyl radicals. Along with, it produces excited H_2O_2 molecules and it is known for UV radiation which also helps in sterilization.

Device B

This device operates at 13.56 MHz frequency. Here, two equi-potential electrodes are at kept at a distance less than the electron-neutral collision mean free path. In device B, equi-potential surface is created by a spiral-cut groove (∼300 μm) on a SS sheet where both sides of this groove are at equal potential. The spiral-cut disc acts as the live electrode and gas flowing out through these grooves generate plasma. A brass disc with 3 mm diameter drilled holes acts as the ground electrode. A schematic of experimental set-up for sterilization using this device is shown in Fig. 26.1b which also shows photograph of the device B. Detailed experimental conditions are shown in table 26.1.

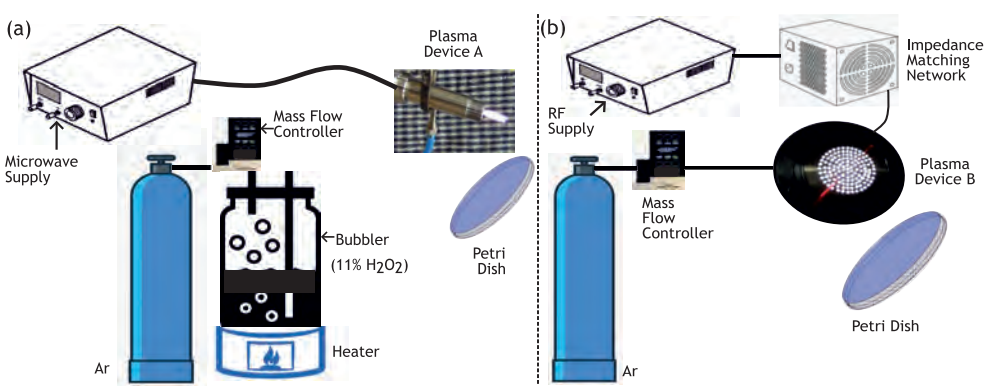

Figure 26.1: Schematic layouts of experiments with plasma devices: (a) Device A & (b) Device B.

26.3 Understanding the Process of Plasma Sterilization

The results of cold plasma treatment on *Aeromonas* bacteria and its bacteriophage is shown in Fig. 26.2. Control sample of this treatment is seen in Fig. 26.2a and Fig. 26.2b shows the conditions of the bacteria just after 2 mins of cold plasma treatment from 6 cm distance (3rd quadrant in Fig. 26.2b). It is seen that cold plasma treatment from device A effectively destroys bacteria. A similar eradication can be achieved by device B after 3 minutes' treatment from 4.5 cm distance (Fig. 26.2c). Figures 26.2d & 26.2f are the controls of bacteriophage

Table 26.1: Experimental details with both devices.

Device	Organisms	Flow details	Operating power (watts)	Duration of exposure (Minutes)	Distance from plasma (cm)
A	*Aeromonas*	10 LPM Ar passed through H_2O_2 (11% solution at 75 °C)	50	1	4.5
				2	6
	Bacteriophage	-do-	-do-	2	6
B	*Aeromonas*	Ar (18 LPM)	60	3	1
	Bacteriophage	-do-	90	4	2

(virus) for devices A & B respectively. For preparation of the control, 5 μl virus of known concentration virus ($\sim 10^6$ pfu/ml) (pfu, plaque forming unit) is spotted on top of previously spread bacterial lawn. Here, clean area indicates death of the bacteria due to viral activity while the bacterial spots indicate destruction of the virus. Higher virus count in the spotted region causes it to form a confluent zone rather than forming individual plaques. Results of cold plasma treatments on virus by devices A & B are respectively shown by Figs. 26.2e and 26.2g. The experimental parameters are mentioned in Table 26.1. It is seen that both these devices were able to control the viral growth after cold plasma treatment. Bacteriophage has

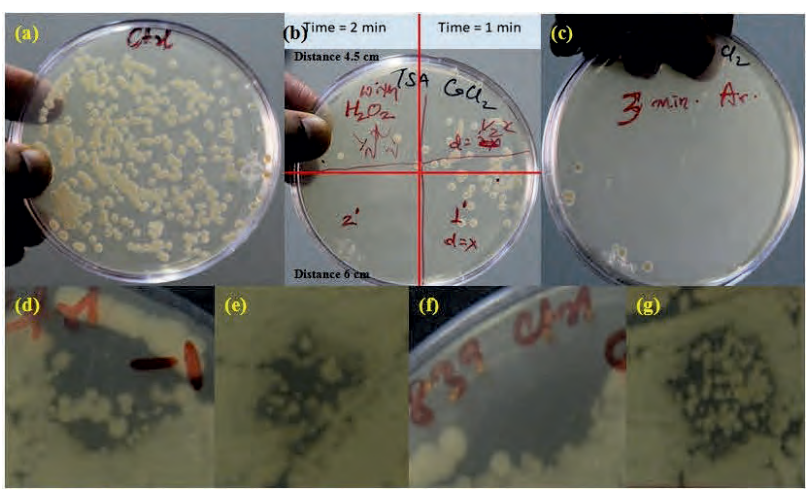

Figure 26.2: (a) Control of *Aeromonas* bacteria, (b) & (c) bacterial growth post 24-hour incubation after cold plasma treatment, respectively with devices A & B, (d) control of bacteriophage (virus) for device A, (e) viral growth post 24-hour incubation after treatment with device A, (f) control of bacteriophage (virus) for device B, (g) viral growth post 24-hour incubation after treatment with device B. Regions marked by circles show clear area due to phage killing from figures (d) to (g).

an outer protective layer made of protein similar to Covid-19 making it very resistant to sterilization process. Researchers also found it stronger than corona virus. The destruction of bacteriophage by these devices thus gives hope that cold plasma devices might also be

effective against COVID-19 virus. They might be useful for sterilization of masks, gloves and reusable medical tools. Figure 26.3 shows OES spectra recorded from both these devices during operation. Figure 26.3a shows emission lines of the different species present in plasma (Device A). Characteristic OH peak at 281 nm & 309 nm emerged here possibly due to dissociation of H_2O_2 in plasma. OH is one of the most important radical for pathogenic destruction. Characteristic H_α line (656 nm), Ar*I* and O*I* lines are also witnessed in this spectrum. It seems that emergence of strongest O*I* line is due to dissociation of H_2O_2 and atmospheric air inside plasma. Presence of O *I*, H_α and OH lines indicate usefulness of the device for pathogenic sterilization. Figure 26.3b shows emission lines of plasma from Device B. Here, OH, O*I*, Ar*I* atomic lines are seen along with NH, NO, N_2 molecular bands in spite of absence of H_2O_2 in plasma. Device B seemingly dissociates ambient atmosphere resulting in emission of all these bands. Reason for this behavior may lie in the principle of different plasma generation mechanisms for these devices. Figure 26.5 shows a typical RF-HC based

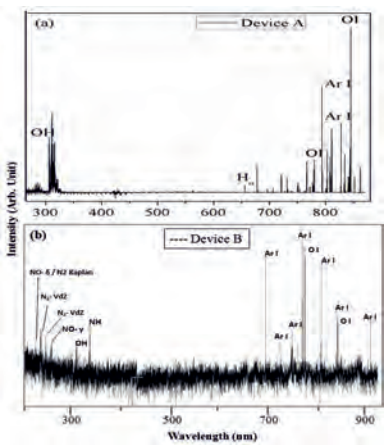

Figure 26.3: Typical OES spectra obtained from devices (a) A & (b) B during operation.

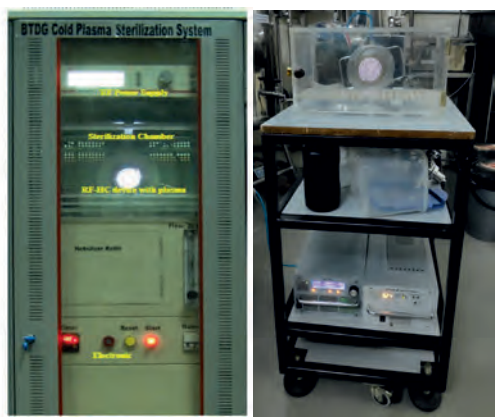

Figure 26.4: Two different Cold plasma sterilization devices.

cold plasma device with all accessories for disinfection of accessories including purse, key ring, cell phones, tiffin box etc. Figure 26.4 shows a more compact version of the device ready to put for everyday use. These Results demonstrate that these cold plasma devices

Figure 26.5: (a) Ongoing cold plasma treatment of N-95 mask, (b) typical cold plasma disinfection set-up, (c) Disinfection of personal belongings under cold plasma treatment.

can eradicate *Aeromonas* bacteria & its phage virus after 3 to 4 minutes of plasma treat-

ment. It is also anticipated that they might have the capability of sterilizing COVID-19 and other similar viral strains. Their sterilization is quicker when compared with the presently available cold plasma-based technologies. Between these two, device B is more suitable for application due to larger area and cost effectiveness as H_2O_2 is not required for sterilization.

Frequently Asked Questions

Q1. How cold plasma helps does sterilization in this particular case?

Q2. What are the bacteria and viruses tested here to show efficacy of the device?

Q3. Compare 2.45 GHz microwave APPJ and 13.56 MHz RF hollow cathode's performances as sterilization device.