Chapter 5

Results and Analysis: Bio-application

5.1 Bacteria Inactivation with DBD

5.1.1 Sterilization Procedure

The DBD system was tested for its sterilization capability and efficiency by exposing three types of bacteria, namely *Escherichia coli, Salmonella enteritidis,* and *Bacillus cereus* to the plasma. The flowchart for the experimental procedure starting from the preparation of bacteria samples performed in the Microbiology Laboratory, Institute of Biological Sciences, to the sterilization treatment performed in the Plasma Research Laboratory, Physics Department, is illustrated in Figure 5. 1.

5.1.2 DBD Discharge Configuration for Sterilization

The two DBD configurations selected for bacteria inactivation as discussed in Section 4.3 are:

"Unipolar" pulsed DBD:	500Hz driving frequency, 1.5mm air-gap, glass dielectric, fixed MOSFET drain voltage, V_{DD}=16V
Sinusoidal voltage DBD:	8.5kHz driving frequency, 1.5mm air-gap, glass dielectric, fixed MOSFET drain voltage, V_{DD}=16V

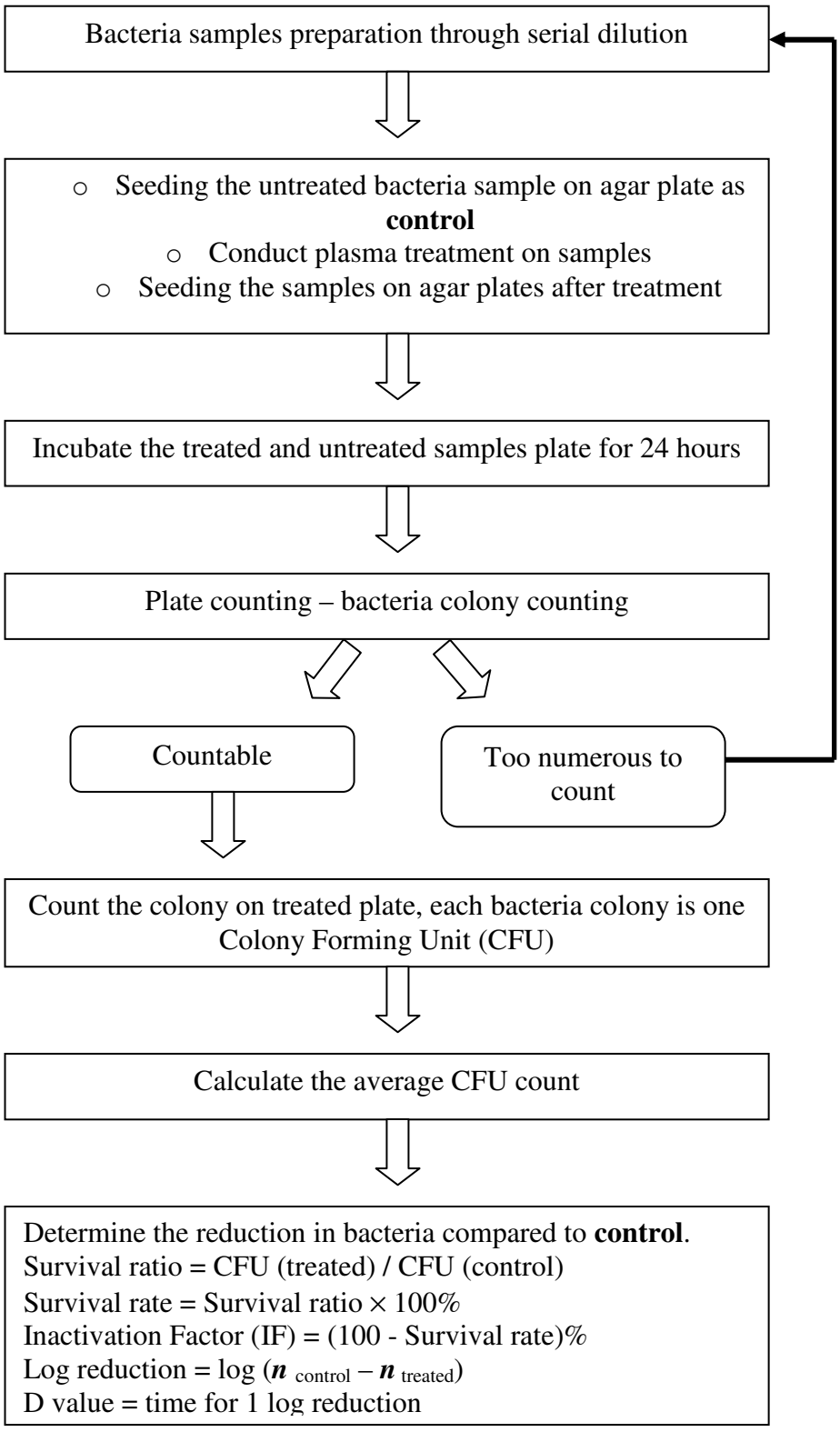


Figure 5.1: Flow Chart of the bacteria sterilization procedure

After a series of preliminary experiments which indicated sinusoidal voltage powered DBD having stronger ability to inactivate bacteria, the time duration* of treatment on the bacteria for each DBD configuration is set as:

”Unipolar” pulses: 15s, 30s, 1min, 2min, 3min, and 4min.

Sinusoidal voltage: 5s, 10s, 15s, 30s, 1min, 2min, and 3min.

* *Longer duration of treatment time may be applied if sterilization cannot be achieved within the specific time. Duration time shorter than 5s is not attempted as it is difficult to handle.*

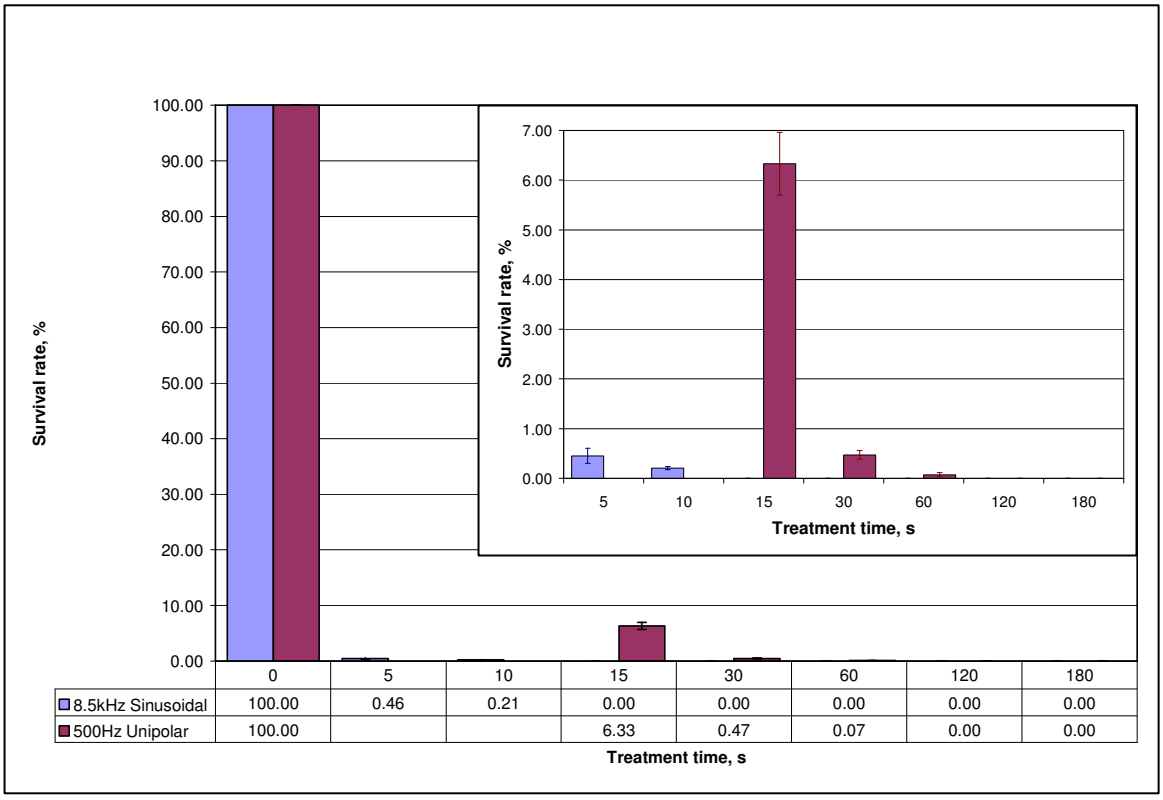
5.1.3 Sterilization through Direct Exposure

Results shows that DBD is effective and efficient in inactivating all bacteria within 1min of direct exposure treatment time in most cases, and the rate depends on the type of voltage excitation. The 8.5kHz sinusoidal voltage is found to be more efficient in sterilizing than the 500Hz “unipolar pulses”. This is due to (i) the amount of UV emission from sinusoidal voltage DBD is more than 5 times higher, and (ii) the microdischarge density in sinusoidal voltage DBD is about 2 times larger.

5.1.3.1 Sterilization of ***Escherichia coli***

For gram negative bacteria *Escherichia coli*, sterilization was achieved between (10-15)s with sinusoidal voltage (Figure 5.2). About 3 log reduction of bacteria amount was recorded, the IF value after 5s of treatment time was more than 99.5. In addition, the D-value (1 log reduction) for *Escherichia coli* was approximately 2s.

However with “unipolar” pulsed DBD, sterilization was only achieved after 1min treatment time. IF value after 15s treatment (shortest treatment) was 93.7, while D-value was approximately 12s.



	0	5	10	15	30	60	120	180
8.5kHz Sinusoidal	100.00	0.46	0.21	0.00	0.00	0.00	0.00	0.00
500Hz Unipolar	100.00			6.33	0.47	0.07	0.00	0.00

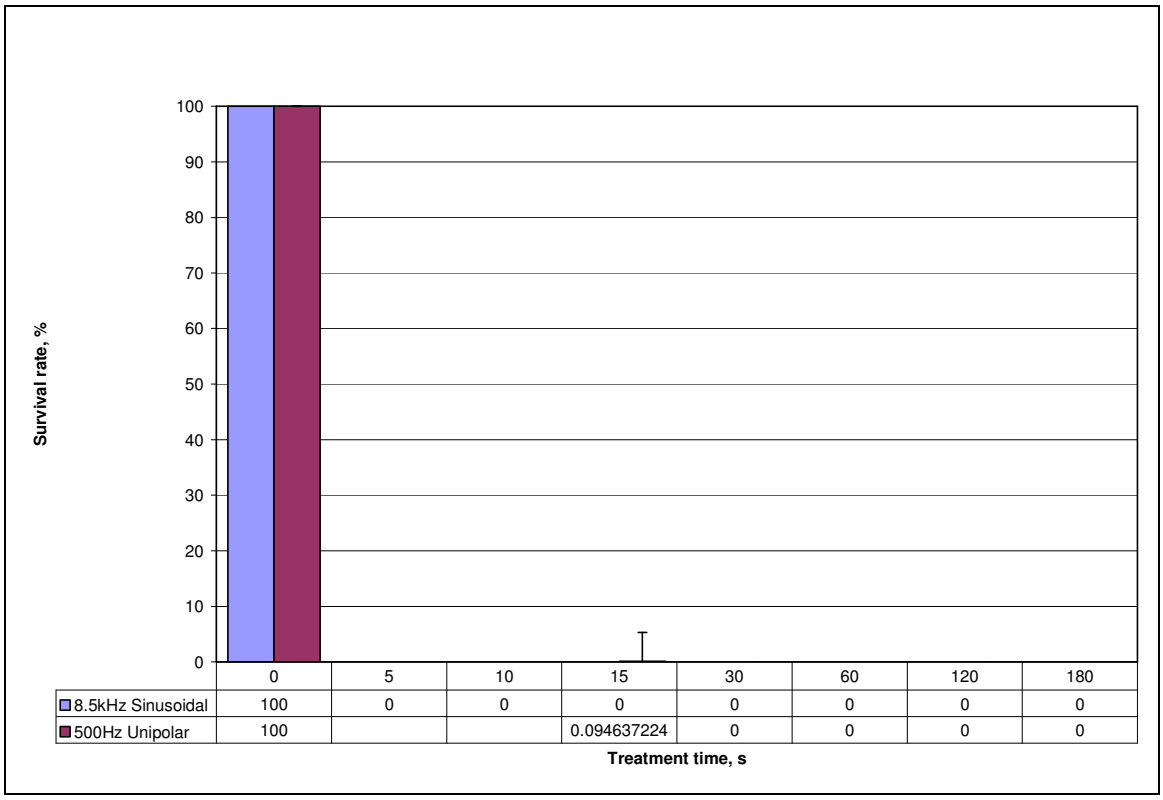
Figure 5.2: Survival rate of *Escherichia coli* against treatment time.

5.1.3.2 Inactivation of ***Salmonella enteritidis***

Another gram negative bacteria *Salmonella enteritidis* recorded complete sterilization within 5s of sinusoidal voltage DBD treatment and 30s for “unipolar” pulsed DBD (Figure 5.3). For sterilization with sinusoidal voltage, more than 3 log reduction in bacteria amount was achieved within the minimum 5s treatment time.

Consequently, the D-value for this case was less than 5s and IF value for the treatment of 5s was 100.

On the other hand, "unipolar" pulsed DBD recorded more than 3 log reduction of bacteria in longer time duration of 30s. D-value was estimated as 5s which is even shorter than the case of *Escherichia coli*. IF value for 15s treatment duration was 99.9.



	0	5	10	15	30	60	120	180
8.5kHz Sinusoidal	100	0	0	0	0	0	0	0
500Hz Unipolar	100			0.094637224	0	0	0	0

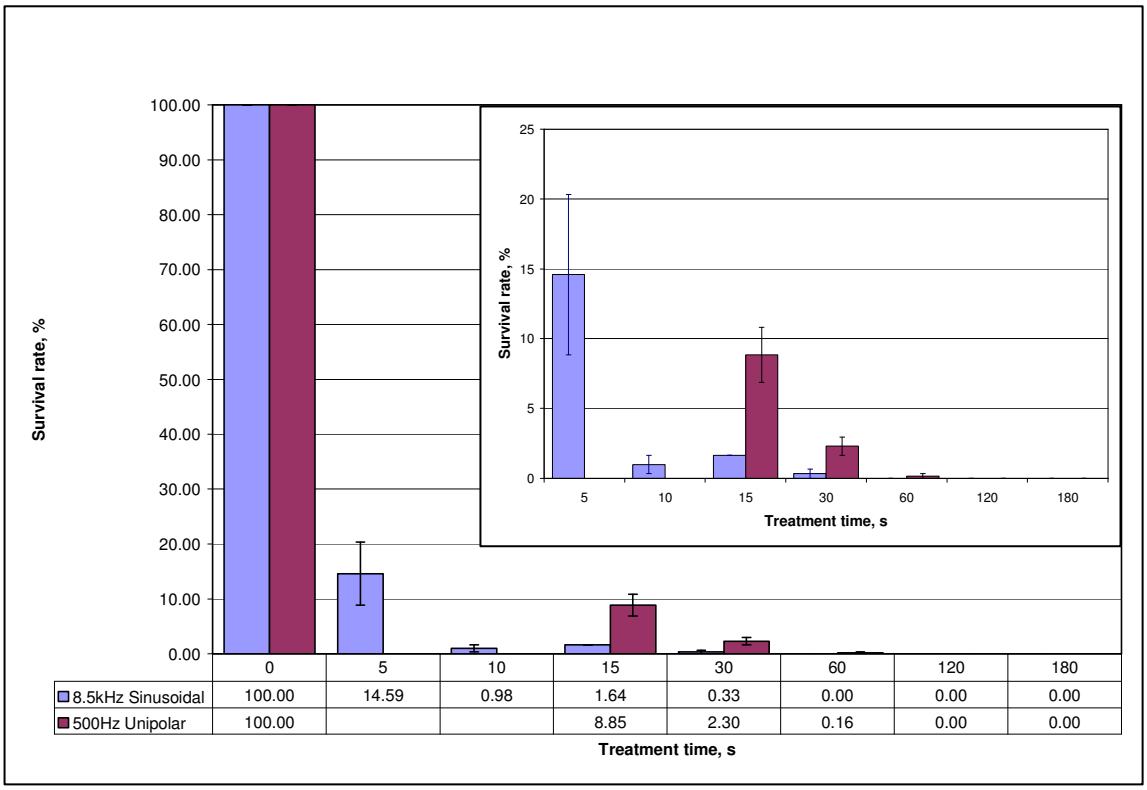
Figure 5.3: Survival rate of *Salmonella enteritidis* against treatment time.

5.1.3.3 Sterilization of ***Bacillus cereus***

For gram positive bacteria *Bacillus cereus*, since the cell wall for this type of bacteria is physically thicker than gram negative bacteria, hence, one would expect the inactivation to take longer treatment time. The experimental results showed that

complete inactivation of *Bacillus cereus* with sinusoidal voltage DBD required between 30s to 1min of treatment time, with D-value of 6s and IF value of 85.4 after 5s treatment time (Figure 5.4).

Sterilization with "unipolar" pulsed DBD recorded complete inactivation within 1min to 2min of treatment time, the longest treatment time encountered of all the conditions investigated; the D-value was recorded as 14s, and IF value reached 91.1 for 15s of treatment time.



	0	5	10	15	30	60	120	180
8.5kHz Sinusoidal	100.00	14.59	0.98	1.64	0.33	0.00	0.00	0.00
500Hz Unipolar	100.00			8.85	2.30	0.16	0.00	0.00

Figure 5.4: Survival rate of *Bacillus cereus* against treatment time.

Tables 5.1 and 5.2 summarize the sterilization results of three types of bacteria treated. For the *E.coli* sample, two different series of treatment with different initial CFU (control) were carried out but the survival rates obtained are similar, hence, the average was calculated for Figure 5.2.

Table 5.1: Summary of sterilization results with sinusoidal voltage DBD

Bacteria Type	Control CFU	Sterilization	D-value	IF after 5s treatment
Escherichia coli	287, 356	(10-15)s	2s	99.5
Salmonella enteritidis	3170	< 5s	< 5s (likely < 2s)	100.0
Bacillus cereus	305	30s – 1min	6s	85.4

Table 5.2: Summary of sterilization results with "unipolar" pulsed DBD

Bacteria Type	Control CFU	Complete Sterilization	D-value	IF after 15s treatment
Escherichia coli	1432, 323	(1-2)min	12s	93.7
Salmonella enteritidis	3170	(15-30)s	5s	99.9
Bacillus cereus	305	(1–2)min	14s	91.1

Pictures of samples of formed colonies of *E. coli* on agar plates at various treatment stages are shown in Figure 5.5. The semi-log plots of the reduction of bacteria (normalized as survival ratio) are shown in Figure 5.6 for treatment under "unipolar" pulses and Figure 5.7 for treatment under sinusoidal voltage. The D-values were deduced from these graphs from the first $\log_{10}$ reduction (1 to 0.1).

There are a few possible bacterial inactivation agents (Laroussi, 2002a; Sun *et al.*, 2007) in the DBD, (i) UV irradiation, (ii) active reactive species and ozone, and (iii) charged particles.

It is known that UV-C (200 to 280nm) and UV-B (280 to 315nm) radiation are lethal to microorganisms. The peak germicidal effectiveness occurs at 264nm (UV-C region) and falls rapidly in the UV-B region to less than 10% at 310nm as shown in Figure 5.8 (Linden and Mofidi, 2004). Referring to Figure 4.32 and Appendix B, most of the significant emission lines from the DBD are in the UV-A region (315 to 400nm). For emission lines ≤310nm, only a few comparatively very low intensity peaks are observed at 295.0, 296.2, 297.68, and 310.4nm (SPS of N_2 molecule) as shown in Figures B.1 and B.2. This indicates UV radiation probably plays a minor role in the sterilization effect observed.

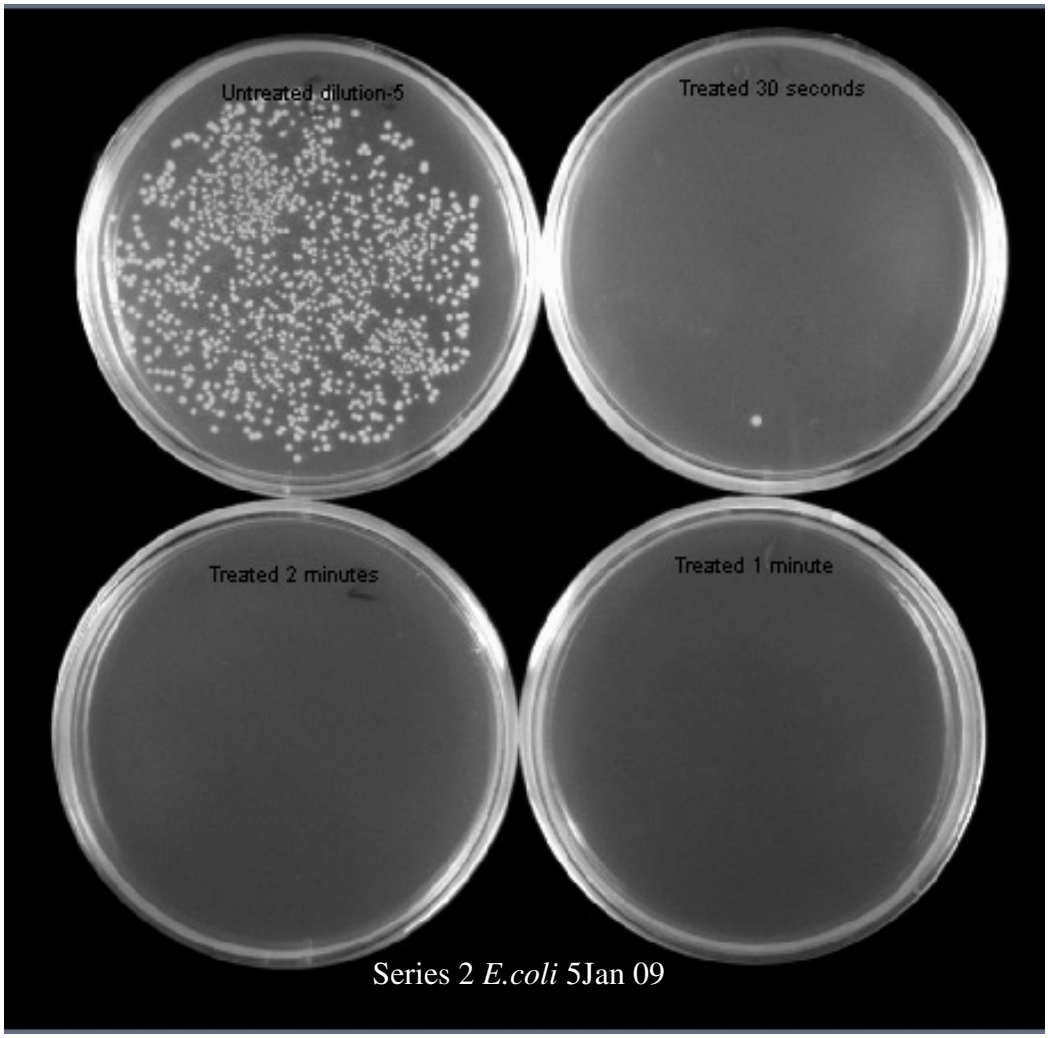


Figure 5.5: Untreated and treated *E.coli* bacteria samples under "unipolar" pulsed DBD plasma cultured in agar plates. (Clockwise from top-left: Control-untreated; treated for 30s; treated for 1min; treated for 2min.)

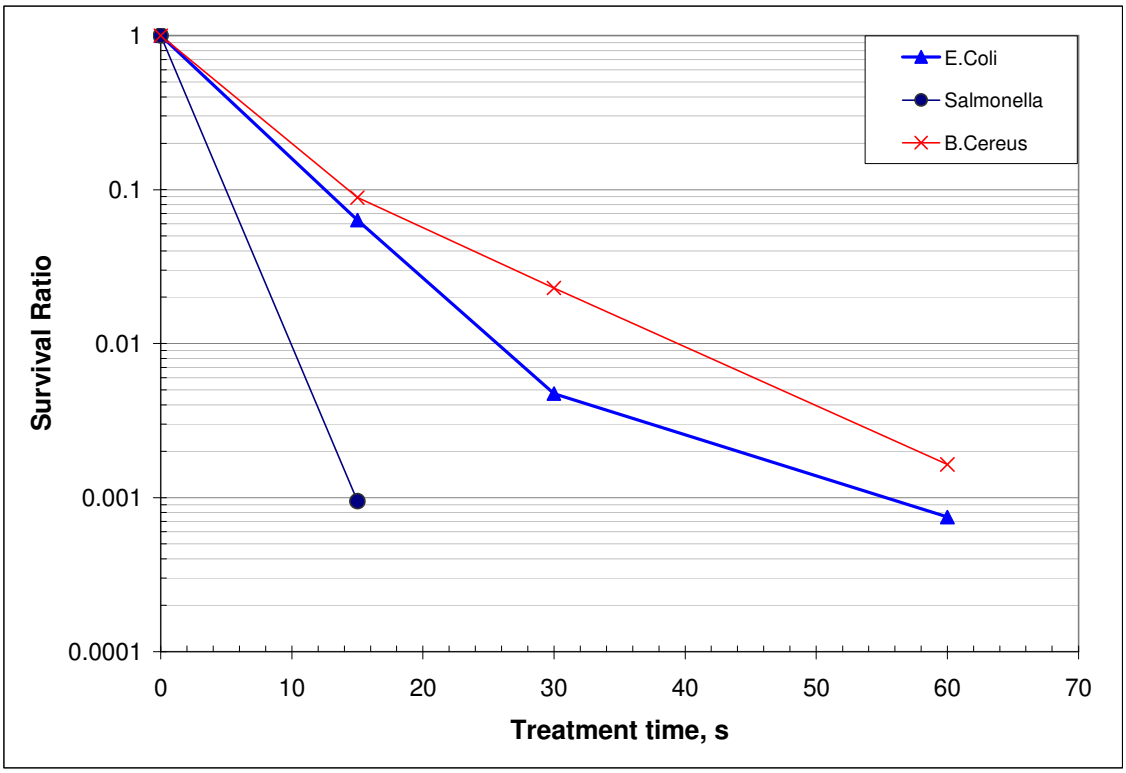


Figure 5.6: Survival ratio against treatment time ("Unipolar" pulsed DBD).

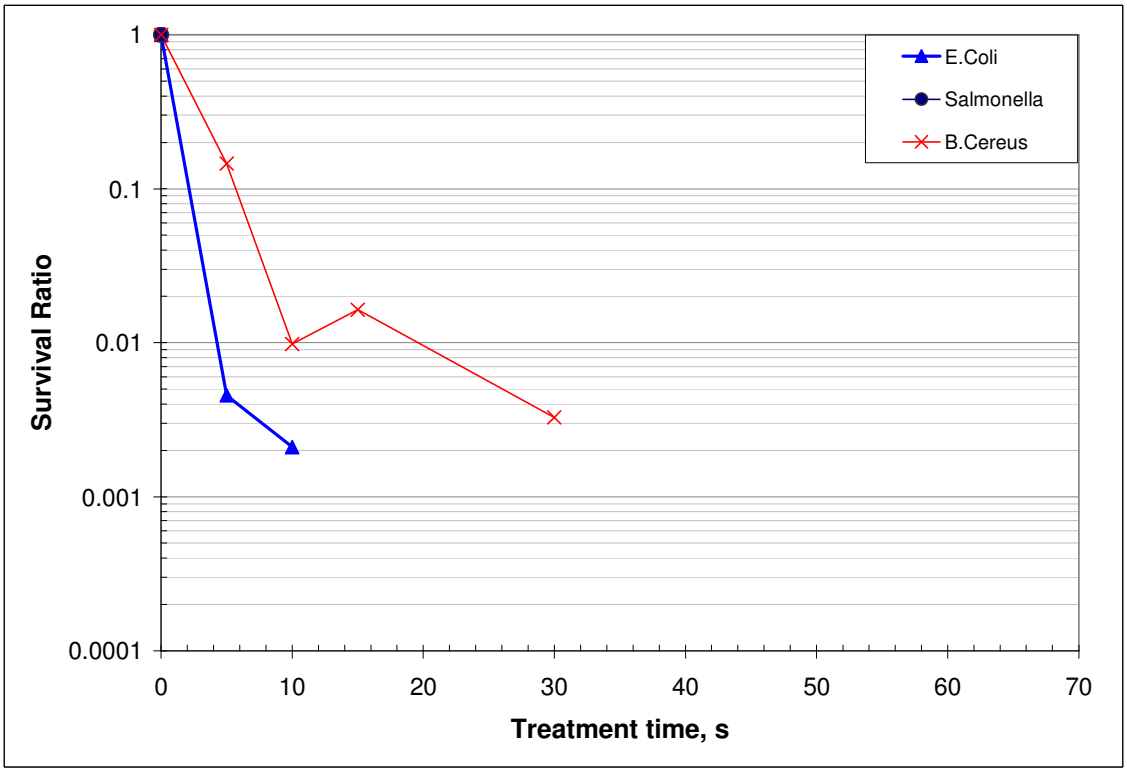


Figure 5.7: Survival ratio against treatment time (Sinusoidal voltage DBD).

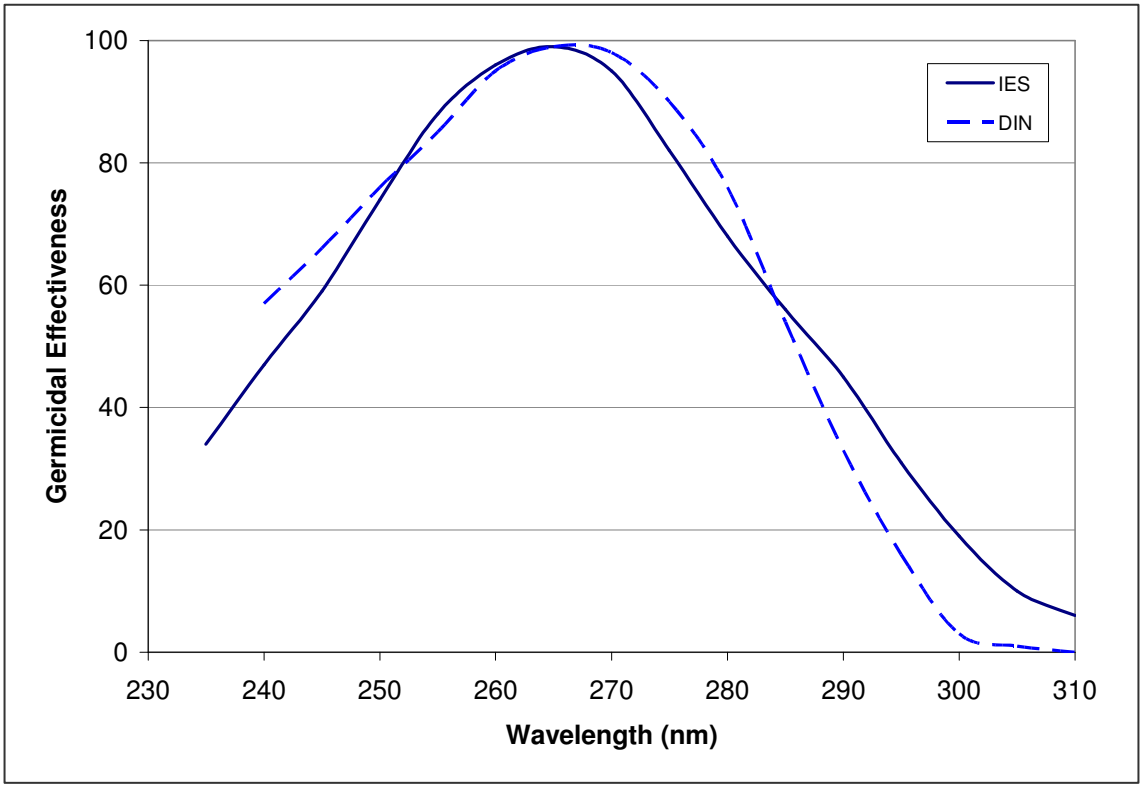


Figure 5.8: The German DIN and IES standard germicidal effective irradiance curves (Linden and Mofidi, 2004).

The existence of some reactive species (e.g. OH, O and O_3) discussed in Section 4.3 would also contribute to the germicidal effect. However, the degree of its contribution could not be estimated as the amount of ozone could not be measured (equipment was not available).

The third deactivation agent, charged particles, can attach to the bacterial cell and electroporates it if the trans-membrane potential difference V_{tm} = 0.7-1V (Dev *et al.*, 2000). The required potential is given as $V_{tm} = 1.5Er\cos\alpha$, where E is the electric field strength, r the cell radius and α the angle between the direction of the field and the cell surface vector. If the applied field strength and pulse-length exceed the upper limit for electroporation, the cell ruptures and damage cannot be repaired. The bacteria used in this investigation have diameter of approximately 1µm, and assuming α = 0,

minimum electric field for electroporation is about 0.7kV/mm which is one order of magnitude less than the applied field. It is deduced that charged particles do play a significant role in deactivation of bacteria.

5.1.4 Sterilization through Indirect Exposure

The effect of sterilization without the bacteria having direct contact with the current filaments was carried out for only the *Escherichia coli* bacteria. *E.coli* is chosen as the target as the slope of the time for complete sterilization is neither too short nor long (Figure 5.6) in comparison. The setup was made by spreading the sample bacteria solution around the glass plate outside the overlapping edge of the electrode area in a ring shape as shown in Figure 5.8. Marking was made on the dielectric to ensure the sample was spread consistently 1cm outside the area where electrodes overlap (area where the plasma is in direct contact). Only the DBD excited by "unipolar" pulses was used. The gap width and drive voltage to the MOSFET, V_{DD}, remained the same.

With this configuration, only the "afterglow" (neutral atoms, radicals, neutral molecules, excited state molecules) and UV radiation can reach the bacteria sample, and direct contact with the plasma current (charged particles) is avoided. The bacteria also avoid the effect of high electric field.

The sterilization results are plotted in Figure 5.10 and it shows that treatment by indirect exposure is less efficient when compared to direct exposure. Complete inactivation treatment time took more than 4min, more than doubled the time for direct contact. The D-value recorded was about 3min compared to 12s (15 times slower) in the previous case of same power supply and gap width configuration (see Table 5.3). This indicates that the reactive species (e.g. O, OH, O_3) in the "afterglow" and the low UV-B radiation do contribute to bacterial inactivation. However, in comparison with the case

of direct exposure to the active plasma column, the effect of charged particles speeds up the sterilization process significantly. Hence, it can be said that the charged particles play a major role in the sterilization effect.

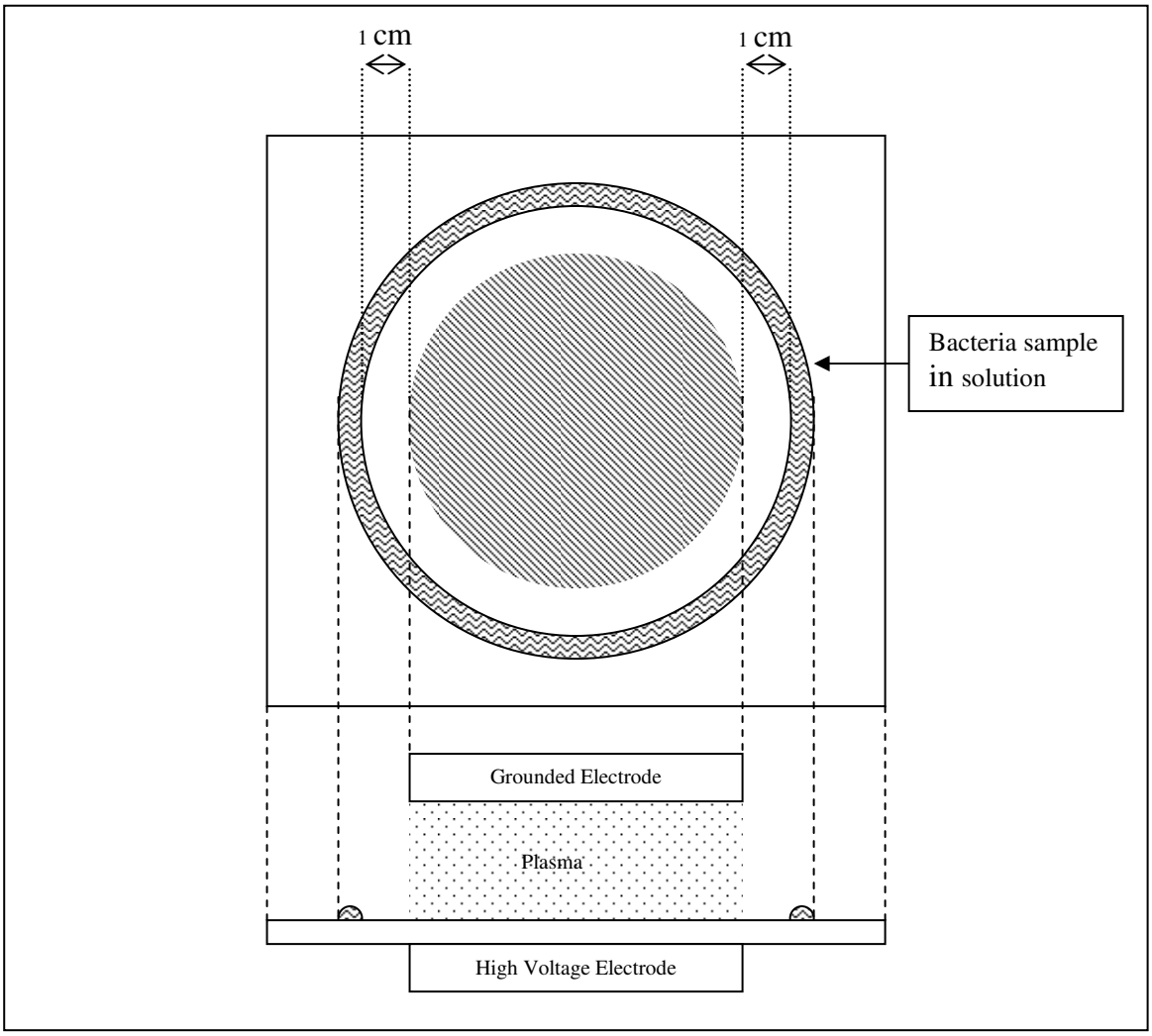


Figure 5.9: Setup of the plasma treatment with sample of 1cm away from plasma column.

Table 5.3: Sterilization performance for direct and indirect exposure of plasma.

Exposure	Complete Sterilization	D-value	IF after 15s treatment
Direct Exposure	(1-2)min	12s	93.7
Indirect Exposure	> 4min	3min	40.5 *(after 30s)

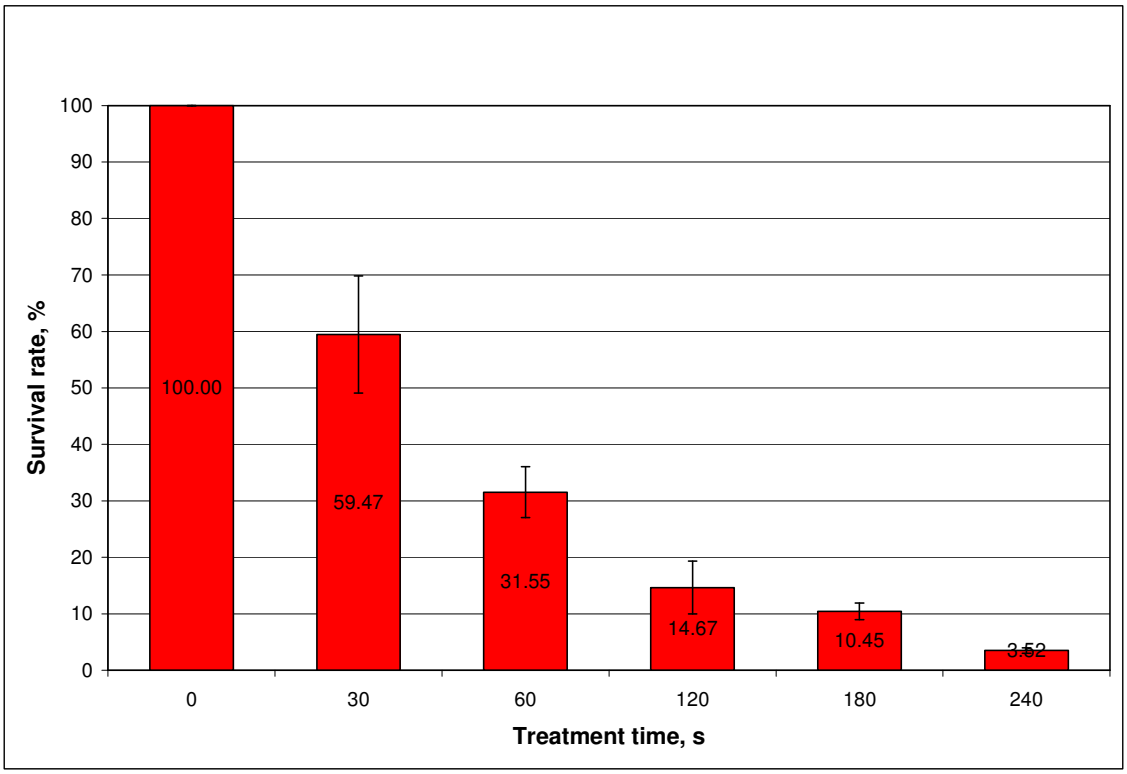


Figure 5.10: Survival rate of *E. coli* treated by indirect exposure to the DBD plasma.

5.1.5 Sterilization with Ground Electrode Insulated

Another experiment was conducted to investigate the effect of the dielectric position to the sterilization efficiency. In the previous cases, the dielectric insulates the high voltage electrode but in this setup, the dielectric barrier is placed to cover the ground electrode. The arrangement is described in Figure 5.11. The results showed no significant change in sterilization efficiency. The complete sterilization time, D-value, IF value recorded is similar to that of the normal setup. This indicates that dielectric position is not a critical factor in sterilization efficiency for the configurations studied.

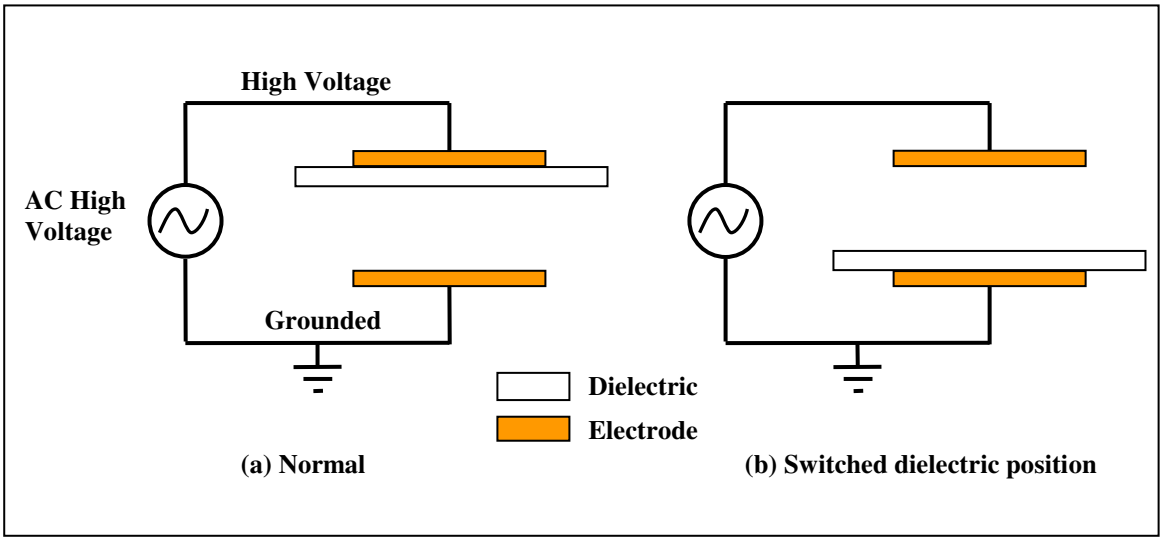


Figure 5.11: Switched placement of dielectric barrier to cover the ground electrode.

5.2 Summary

From the above, it was concluded that the DBD was effective in killing bacteria with complete inactivation in 1min for most cases investigated and 3-log reduction of bacteria CFU was achieved. It is deduced that charged particles in the active plasma column (direct exposure) play more significant role in rapid sterilization when compared to the reactive species and UV radiation. The sterilization effect was not affected by the dielectric position but the power delivered to the electrode is a critical factor. By using sinusoidal voltage excited DBD, sterilization is achieved more rapidly than “unipolar” pulsed DBD since the power density per unit area for sinusoidal voltage is higher than “unipolar” pulses.