Chiang Mai J. Sci. 2018; 45(4) : 1811-1826
http://epg.science.cmu.ac.th/ejournal/
Contributed Paper

Sterilization of Medical Equipment by Plasma Technology

Norrapon Vichiansan [a], Noppakarn Kaai [a], Komgrit Leksakul* [a,b], Saisamorn Lumyong [c] and Jeon Geon Han [b,d]

[a] Department of Industrial Engineering, Faculty of Engineering, Chiang Mai University, Chiang Mai 50200, Thailand.

[b] Thai-Korean Research Collaboration Center (TKRCC), Science and Technology Park.

[c] Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand.

[d] Nu-SKKU Joint Institute for Plasma-Nano Materials, Sungkyunkwan University, Republic of Korea.

* Author for correspondence; e-mail: komgrit@eng.cmu.ac.th

Received: 4 January 2018
Accepted: 30 January 2018

ABSTRACT

This study examined sterilization of scalpels using plasma technology by comparing the performance effectiveness of two techniques, plasma solution (PS) and plasma dielectric barrier discharge (PDBD), on *Escherichia coli* and *Staphylococcus aureus* at 0.5 McFarland. The experimental design was used to determine the optimal condition for both the techniques to be 100% effective by using the ten-fold dilution spread plate count. For PS, the voltage was set at 520 V for 20 s and the concentration of sodium acetate was 1 g/L. For PDBD, the distance between the anodes was 9.4 mm; the discharge time was 14 min; the power was 76 W; and the argon gas flow rate was 22 L/min. On comparing the time taken for 100% sterilization by the two techniques, it was found that PS used less time than PDBD. However, upon testing with Optical Emission Spectrometry (OES), the hydroxyl free radicals produced by PDBD were observed to be greater than those produced by PS.

Keywords: solution plasma, PDBD, *Escherichia coli*, *Staphylococcus aureus*, OES

1. INTRODUCTION

Sterilization is a widely used approach to remove microorganisms that contaminate the surface of various equipment. Such microorganisms include bacteria, virus, fungi, and microplasma, and some are classified as beneficial microorganisms for humans. However, some species cause serious and life-threatening diseases. As a result, sterilization is crucially important, and there are many researchers interested in this topic. There are several methods of sterilization, such as chemical [1] and physical [2] methods. Each method has as its ultimate goal the minimization of the number of microorganisms. In addition, many researchers have also focused on finding a highly effective method for sterilization by using new advanced technologies.

A review of the incidence of infections among patients in hospitals during surgery shows that most of them are caused by external contamination, from the laboratory

[3]. Sterilization, thus, plays an important role in reducing the risk of infection from microorganisms in these patients' lives. The most popular sterilization is autoclave process adopted at present is complicated and time-consuming; each sterilization requires at least 30 min, which does not include the time required for the temperature to reach as high as 121.5°C. As might be expected, such high temperatures cause damage to devices with thermal corrosion on stainless steel devices that might be occurred by fatigue effect.This effect can made the infection that can take patients life. As a result, it becomes necessary for various organizations to purchase many replacement devices to meet their needs. If the damage, as well as the processing time, of the sterilization process can be reduced, it will result in cost reduction. With regard to current technologies, plasma technology can be used as a technique to solve the problem effectively as it has been shown in recent research that plasma technology was used to sterilize *E. coli* and *S. aureus* and that it could reduce the processing time of sterilization to around 20-50 s compared to the conventional technique used by hospitals. [4] This technology improves the efficiency of the sterilization process and solves the equipment rotation problems that occur in hospitals remarkably well.

Plasma-activated water or PAW [1, 6] is liquid which contained very high reactive species, Use of PAW is an effective chemical physiological method to produce free radicals in water for seed germination, pesticide breakdown, water treatment, and sterilization. A research study on the behavior of plasma on addition of an electrolyte solution for low voltage power supply found that the electrical conductivity, as in the study revealed that the optimal process of Plasma Solution (PS) for the optimal voltage to produce hydroxyl free radicals should be in the range of 400-600 microsiemens per cm, as well as that the suitable solutions to be used in sterilization include potassium chloride (KCl), sodium chloride (NaCl), sodium sulfate (Na_2SO_4), and sodium acetate (CH_3COONa) [7]. In contrast, a study by Takai found that using potassium chloride and sodium chloride solutions produced ClO ions which could eliminate microorganisms, whereas sodium sulfate and sodium acetate solutions had no effect on microorganisms when tested in PS [4].

Sterilization using the Plasma Dielectric Barrier Discharge (PDBD) technique, powerful cool gas plasma, can produce plasma with stability. Moisan and Wertheimer compared two types of plasmas, microwave and radio frequency (RF), using the PDBD technique [8]. Feichtinger et al., (2003) developed plasma solution for sterilization using PDBD at low pressure [9]. Liu et al. (2008) studied the effects of plasma spacing on sterilized specimens for sterilization [10]. Schneider (2013) achieved a reduction in the sterilization time at low temperatures [11]. Korachi et al. (2010) studied the effects of plasma on pH, and Schneider et al. (2005) studied the use of cold plasma sterilization at industrial plants [12, 13]. Cold plasma is a technique commonly used in current research because it does not damage materials with low melting points [14-16]. According to the literature review can showed the possible effective of plasma to sterilized microorganisms unless it still not be 0 cfu but in the future it might be sterilized until sterilization state.

On reviewing related literature, it was found that there were no studies comparing the plasma techniques PS and PDBD in sterilization. The two techniques have different abilities in producing free radicals, especially with regard to the free radical

production of hydroxyl, which is a highly severe oxidizing agent. PDBD produces free radicals from air vapor while PS produces them directly from water. Therefore, this study aimed to compare the efficiency of *E. coli* and *S. aureus* sterilization on medical equipment using hydroxyl radicals produced by the two plasma techniques in order to seek the possibility of applying the techniques properly or as a new alternative for medical equipment sterilization.

2. MATERIAL AND MEDTHODS

2.1 PS Sterilization

The PS generator is a DC power supply that can provide a maximum voltage of 900 V at 50 Hz with 2.4 mm tungsten rods as electrode poles [4]. Dip the electrode poles from the top of the solution container. The cathode pole is dipped 5 mm in the solution, measuring from the surface. The anode pole is dipped 15 mm, which is deeper than the cathode pole. The two poles are 10 mm apart from each other, as shown in Figure 1. In the PS sterilization process, a magnetic bar is used to stir the solution and the microorganisms are stirred with a magnetic stirrer. The speed is constantly adjusted to 150 rpm for all conditions of the experiment.

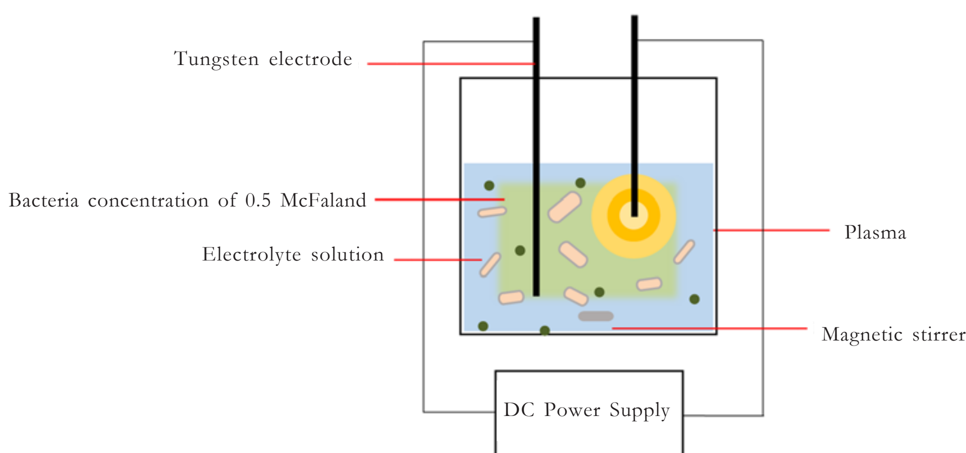


Figure 1. Components of the PS experiment.

2.2 Experimental Design

In the experimental trial, the following factors were considered for PS sterilization on medical equipment: voltage, discharging time, type of solution (sodium sulfate :Na_2SO_4 and sodium acetate : CH_3COONa solution) and solution concentration. In this experiment, Na_2SO_4 and CH_3COONa solution were chosen, because they won't produce negative ion which can kill microbial before plasma discharged. That could be the noise of the experiment. However, DI water couldn't generate plasma with low voltage discharged. Table 1 presents the lowest voltage used, that of 520 V (this was the lowest voltage that could produce plasma), and the highest level was 600 V due to higher power would have melted the electrodes. In order to find the optimal parameters for PS sterilization, the experiment conditions were designed base on 3 factors with each using 2^3 experimental designs, or eight conditions. Each condition was tested two times for Na_2SO_4 and CH_3COONa solution. At the midpoint (560 V, 40 S, 1.5 g/l), this condition was repeated three times. Consequently, there were 19 experiments for each solution.

Table 1. Factors used to study optimal range of PS sterilization for Na_2SO_4 and CH_3COONa solution.

Factor	Low (-1)	Mid (0)	High (+1)	Symbol
Voltage (V)	520	560	600	A
Time (min)	20	40	60	B
Concentration of solution (g/L)	1	1.5	2	C

When optimal condition of the sterilization was obtained, the experiment was conducted to confirm the results of the sterile condition in the solution system. Sample of scalpel handles dipped into the solution contaminated with *E. coli* and *S. aureus*. Then, the solution and the scalpel handles were made to undergo plasma treatment and were examined to determine the extent of bacterial contamination on the culture plate by using the ten-fold dilution spread plate technique.

2.3 PDBD Sterilization

A PDBD generator uses Plasma's 600 W, 13.56 MHz Radio Frequency (RF) transmitter featuring a slim stainless steel workpiece that can move in and out. The electrodes are made of dielectric stainless steel from alumina ceramics (Al_2O_3). The distance between the electrodes can be adjusted by up to 5 mm, and it can supply three types of gas: argon, oxygen, and nitrogen but in this experinment we focused only on 2 gases: argon and oxygen. Figure 2 presented the set up used for scalpels PDBD sterilization technique.

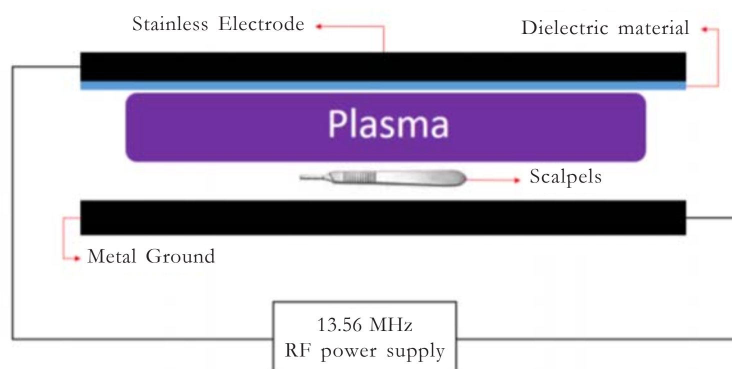


Figure 2. Components of the PDBD experiment.

2.4 Experimental Design

The experiment of PDBD sterilization of medical equipment consisted of five factors: distance between the anode and the scalpel handles, discharge time, voltage, type of gas, and gas flow rate, as shown in Table 2. In addition, the experiment aimed at determining the optimal parameter for PDBD sterilization. The experimental design was divided into two sets. Each set used a $^1/_4$ fractional factorial design, and there were 8 (2^{5-2}) experimental design conditions, each of which was tested four times. There was also one condition at the midpoint (4.4 mm, 7.5 min, 130 W, Ar/O_2, 16 L/min). Each was tested four times, total 36 experiments.

Table 2. Factors used to determine optimal range of PDBD sterilization of medical equipment.

Factor	Low (-1)	Mid (0)	High (+1)	Symbol
Distance between plasma anode and scalpel handles (mm)	3.8	4.4	5	A
Processing time (min)	5	7.5	10	B
Power (Watt)	80	130	180	C
Type of gas	Ar	Ar, Ar/O_2	Ar/O_2	D
Gas flow rate (l/min)	10	16	22	E

2.5 Optical Emission Spectroscopy Testing

Optical Emission Spectroscopy (OES) AvaSpec 2048 Miniature Fiber Optic Spectrometer - was used to identify the reactive plasma species. It was operated with a full range fiber optic and the results were analyzed with the AvaSoft program. The optical emission spectra were recorded in a wavelength range of 200 to 800 nm. OES was used to measure the intensity of light at the wavelength of 309 nm, which was the wavelength of the hydroxyl free radicals derived from PS and PDBD in each of the conditions. After that, using the free radical graph, the hydroxyl free radicals derived from the use of Na_2SO_4 and those from the use of CH_3COONa to produce PS were compared. Figure 3 and 4 presented the schematic diagram of OES measure on the PS and PDBD plasma system.

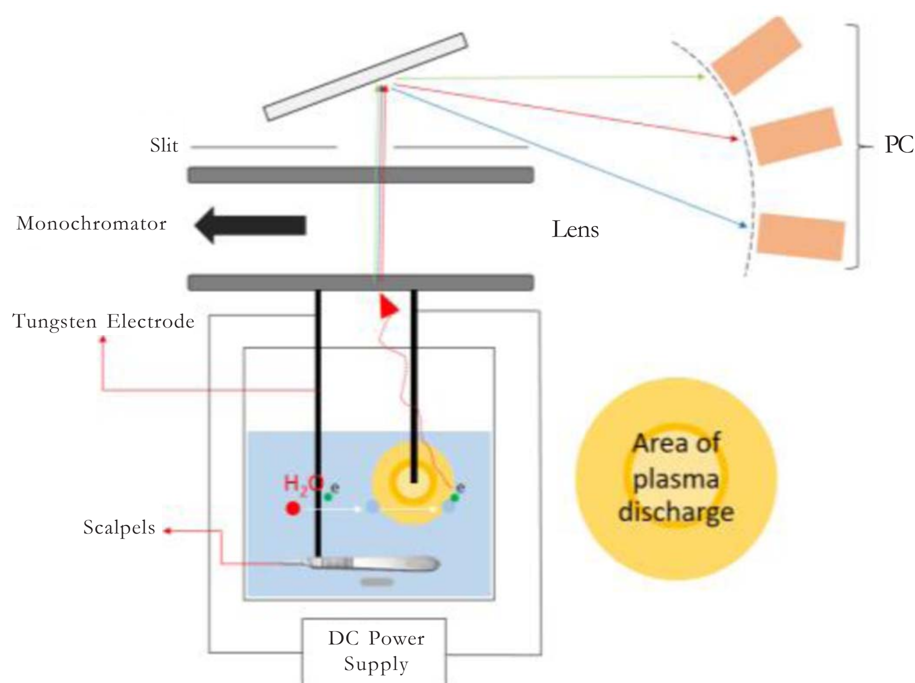


Figure 3. Schematic diagram of OES measured on the PS plasma system.

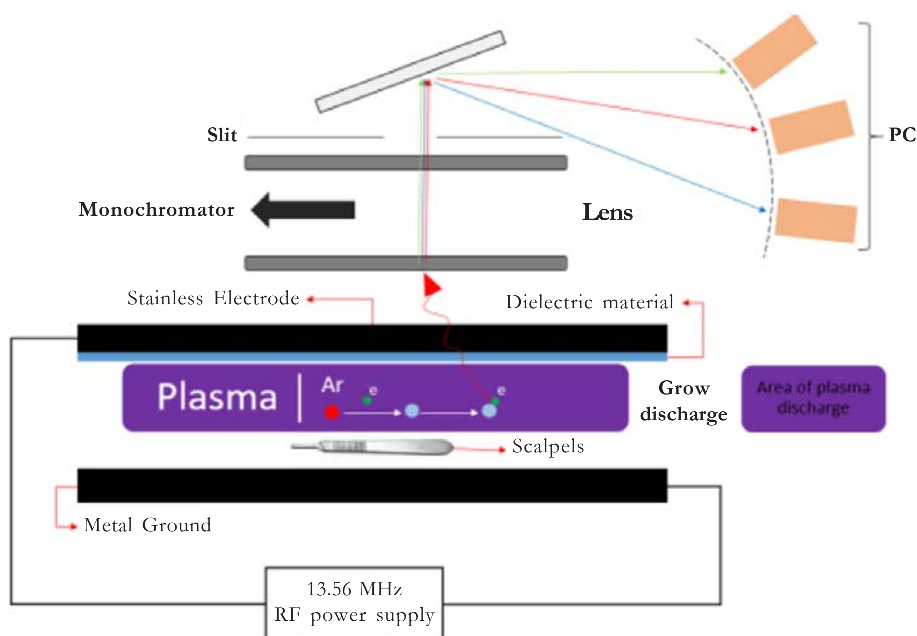


Figure 4. Schematic diagram of OES measured on the PDBD plasma system.

2.6 Preparation of *Escherichia coli* and *Staphylococcus aureus*

E. coli and *S. aureus* were prepared in a rose apple-shaped bottle cultured with nutrient broth (NB), 5 g peptone, and 3 g beef extract per 1 L of water. Then, it was shaken for 12 hr [17]. Dropping bacteria directly on scalpel handles to create biofilm could not be conducted since the concentration of the bacteria would be too low. Therefore, the preparation of the scalpel handles to be tested by using the PS technique could be done as follows. Put the culture medium into 700 ml D.I. sterilized water. Add the bacteria and adjust the number of bacteria by measuring the concentration, 0.5 McFarland, and by detecting the spectral wave at a wavelength of 600 nm. For the PDBD technique, add the bacteria at a concentration of 0.5 McFarland on the scalpel handles, around 5 ml, and leave to dry in a curing room at the temperature of 37 °C for 4 hr for plasma sterilization test.

2.7 Microorganism Inspection

After PS and PDBD sterilizations, the bacteria were inspected by using the ten-fold dilution spread plate technique. The sample solutions, 10^0 to 10^{-6}, were diluted with 10 g peptone solution and 5 g/L sodium chlorate per 1 L of water. A volume of 100 μL of the diluted solution was added into the nutrient agar (NA) in a petri dish of 5 g peptone containing 3 g/L beef extract and 20 g/L jelly powder per 1 L of water. Thereafter, the culture plates were spread. The plates were then incubated at 37 °C for 24 hr. After that, the number of colonies that had grown on the agar was counted; it was compared to the control set, and the concentration of bacterial growth (log 10) was reported and compared to the initial concentration of the bacteria. Calculation for the percentage of inhibition of the bacterial growth was then carried out in order to obtain the optimal parameter possible to inhibit the growth of the bacteria. The acceptable number of bacteria was 0 CFU.

3. RESULTS

3.1 Experimental Results of PS Treatment for *E. coli* and *S. aureus*

Experimental results of PS treatment for *E. coli* and *S. Aureus* by generating plasma discharge with Sodium Sulfate and Sodium Acetate. Experimental conducted based on full factorial design and all results were reported in Table 3.

In the experiment of *E. coli* and *S. aureus* sterilization by using the PS of sodium sulfate and sodium acetate, as shown in Table 3, when the experiment results were calculated by using the Mini-tab 16.1 program, it was revealed that all the factors in the experiment were significant. In addition, the R-squared value was 99.95%, as shown in Table 4, which means that the equations derived from this experiment was significant and could apply for response prediction; the residual graph was a normal distribution as well. There is also distribution of data at the same point as the data of the sodium sulfate solution shown in Figure 5, which could formulate the equation as follows.

$$Y = 77.77 + 14.29A - 10.12B - 18.18C + 2.18AB - 10.23AC - 14.18BC + 6.24ABC \quad (1)$$

According to Eq. (1), the conditions yielded the result of the experiment Y as close to 100%. There were two conditions: the first was A = -1, B = 1, C = -1 and the second was A = 1, B = -1, C = 1. Both these conditions gave 99.94% sterilization of the microorganisms. However, the second condition used less power and concentration for sterilization, A = -1 or 520 V and C = -1 or equal to 1 g/L. As a result, this condition is rather economical and suitable for the sterilization of *E. coli* and *S. aureus*.

Table 3. Experimental results of PS with full-factorial experimental design of sodium sulfate solution and sodium acetate solution.

Voltage (V)	Time (s)	Concentration (g/L)	Average Percentage of *E. coli* Sterilization		Average Percentage of *S. aureus* Sterilization	
			Sodium Sulfate	Sodium Acetate	Sodium Sulfate	Sodium Acetate
520	20	1	83.78	100	100	100
520	20	2	67.78	100	100	100
520	60	1	100	100	100	100
520	60	2	2.35	100	100	100
600	60	1	100	100	100	100
600	60	2	68.23	100	100	100
600	20	1	100	74.33	100	100
600	20	2	100	72.48	100	100
560	40	1.5	100	100	100	100

Table 4. Coefficient analysis of eliminated microorganism percentage using sodium sulfate and sodium acetate plasma solutions.

	Term	Coefficient	SE Coefficient	T	P	R-sq.	R-sq. (adj.)
Sodium Sulfate	A	14.29	0.2223	64.27	0.000	99.95%	99.92%
	B	-10.12	0.2223	-45.53	0.000		
	C	-18.18	0.2223	-81.75	0.000		
	AB	2.18	0.2223	9.81	0.000		
	AC	10.23	0.2223	46.03	0.000		
	BC	-14.18	0.2223	-63.77	0.000		
	ABC	6.24	0.2223	28.05	0.000		
	Ct Pt	22.23	0.5595	39.73	0.000		
Sodium Acetate	A	-6.649	0.1495	-44.48	0.000	99.84%	99.71%
	B	6.649	0.1495	44.48	0.000		
	C	-0.231	0.1495	-1.55	0.153		
	AB	6.649	0.1495	44.48	0.000		
	AC	-0.231	0.1495	-1.55	0.153		
	BC	0.231	0.1495	1.55	0.153		
	ABC	0.231	0.1495	1.55	0.153		
	Ct Pt	6.649	0.3762	17.68	0.000		

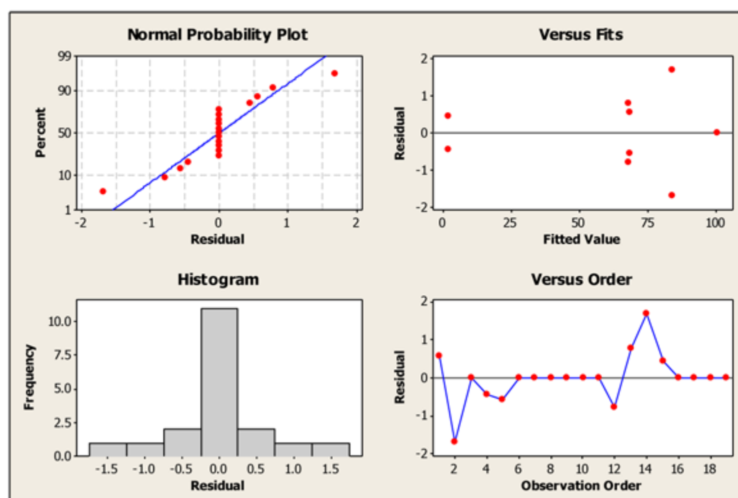


Figure 5. The distribution chart of the sodium sulfate PS Mini-tab program.

Regarding the result of *E. coli* and *S. aureus* sterilization using sodium acetate solution, which is shown in Figure 5, it could sterilize 100%. The calculation result using the mini program 16.1 shown in Table 4 indicates that the significant factors are A, B, and AB. The R-squared value was 99.84%. This means that the equation derived from this experiment is appropriate for use in real situations. The residual graph is a normal distribution as well. There is also distribution of data at the same point as the data of

sodium acetate shown in Figure 3. This can be used to write the equation as follows.

$$Y = 93.351 - 6.649A + 6.649B - 0.231C + 6.649AB - 0.231AC + 0.231BC + 0.231ABC \quad (2)$$

According to Eq (2). the condition that yields the results of the Y experiment close to 100% is A = -1, B = -1, and C = -1 s, or 520 V for the period 20 s and concentration of 1 g/L. The results showed 100% microorganism of *E. coli* and *S. aureus* sterilization, as shown in Table 3. This reveals that the data of the residual graph is a normal distribution except for the fact that the data had duplicate points with a single value at one of the values of the graph. However, when considering the four graphs in Figure 6, the obtained equations can be used and are sufficient for the application microorganism of *E. coli* and *S. aureus* sterilization.

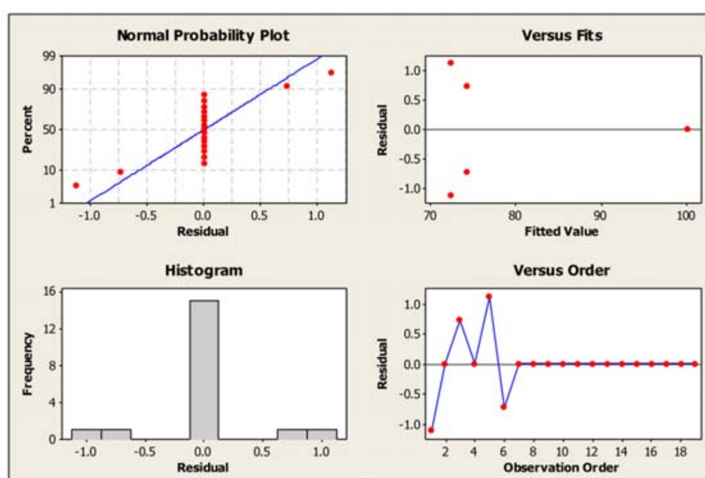


Figure 6. The distribution chart of the sodium acetate PS Mini-tab program.

Optimal conditions were selected and conducted to prove the efficiency of sterilization, the parameters were set and the results were shown in Figure 7. Sodium sulfate and sodium acetate were used in generating the plasma solution. Then, they were tested with scalpel handles which were placed into a container contaminated with *E. coli* and *S. aureus* bacteria. When the contaminated solutions and the scalpel handles were inspected after the plasma treatments, and the microorganisms on the PDA plates were counted. The 100% sterilized were reported, meaning that the scalpel handles were no longer microorganism contaminated.

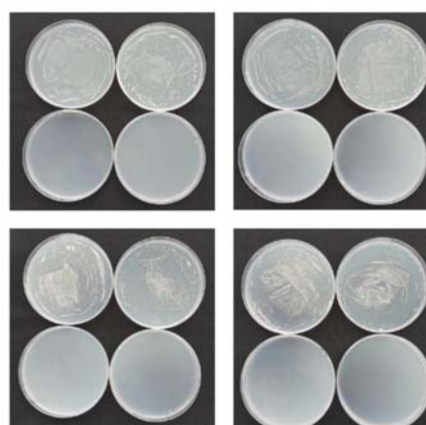


Figure 7. The culture test results for the optimal parameters of sodium acetate (left) and sodium sulfate (right) against *Escherichia coli* (upper) and *Staphylococcus aureus* (bottom) on the scalpel handles.

3.2 Experimental Results of PDBD Treatment for *E. coli* and *S. aureus*

Experimental results of PDBD treatment for *E. coli* and *S. Aureus* by generating plasma discharge with Argon and Oxygen gas. Experimental conducted based on full factorial design and all results were reported in Table 5.

As regards the results of the experiments of sterilization of *E. coli* using PDBD, shown in Table 5, calculations were carried out after the experiments by using the Mini-tab 16.1 program, and it was found that all of the factors used in the experiment were significant and that the R-squared value was 88.35%, as shown in Table 6, meaning that the equation was appropriate for use in real situations. In addition, the residual graph was a normal distribution, as shown in Figure 5. The equation of the experiment can be written as follows.

$$Y = 6.01A + 29.48B - 8.13C - 12.52D + 16.35E - 23.27AB - 13.32AC + 10.84AE + 10.15BC + 22.62ABC \quad (3)$$

The response optimizer function can be used to find the conditions that yield the result of the Y experiment closest to 100% A = 0.896030, B = 0.417054, C = -0.951565, D = -1, and E = 1.

Table 5. Results of PDBD experiment by fractional factorial design of *E. coli* and *S. aureus*.

Distance between Plasma Anode and Scalpel Handles (mm)	Processing Time (min)	Power (Watt)	Type of Gas	Gas Flow Rate (l/min)	Average Percentage of Bacterial Incubation by PDBD	
					E. coli Sterilization	*S. aureus* Sterilization
3.8	5	80	Ar/O_2	22	31.53	46.4
5	5	80	Ar	10	100	69.44
3.8	10	80	Ar	22	100	100
5	10	80	Ar/O_2	10	46.86	75.82
3.8	5	180	Ar/O_2	10	90.91	100
5	5	180	Ar	22	58.34	66.52
3.8	10	180	Ar	10	85.43	81.37
5	10	180	Ar/O_2	22	100	100
5	10	180	Ar	10	69.51	71.07
3.8	10	180	Ar/O_2	22	100	100
5	5	180	Ar/O_2	10	33.22	34.99
3.8	5	80	Ar	10	59.22	42.07
5	10	80	Ar	22	100	25.78
3.8	10	80	Ar/O_2	10	100	100
5	5	80	Ar/O_2	22	100	100
3.8	5	80	Ar	10	38.02	53.2
4.4	7.5	130	Ar	16	100	100
4.4	7.5	130	Ar/O_2	16	40.99	50.36

Table 6. Coefficient analysis of *E. coli* and *S. aureus* percentages eliminated by PDBD.

Microorganism	Term	Coefficient	SE Coefficient	T	P	R-sq.	R-sq. (adj.)
E. coli	A	3.00	22.98	1.31	0.207	88.35%	78.54%
	B	14.74	22.98	6.41	0.000		
	C	-4.06	22.98	-177	0.093		
	E	-6.26	22.98	-2.89	0.009		
	AB	8.17	22.98	3.56	0.002		
	AC	-11.64	22.98	-5.06	0.000		
	AD	-6.66	22.98	-2.90	0.009		
	AE	-2.62	22.98	-1.14	0.269		
	BC	5.42	22.98	2.36	0.029		
	BC	5.07	22.98	2.21	0.040		
	BD	2.34	22.98	1.02	0.321		
	BE	4.10	22.98	1.78	0.090		
	ABC	11.31	22.98	4.92	0.000		
	ABD	-2.04	22.98	-0.89	0.387		
	ABE	3.21	22.98	1.40	0.178		
	Ct Pt	-2.49	6.893	-0.36	0.722		
S. aureus	A	-9.93	-4.96	2.692	0.081	81.05%	65.08%
	B	17.68	8.84	2.692	0.004		
	C	3.17	1.59	2.692	0.563		
	E	10.90	5.45	2.692	0.045		
	AB	-0.64	-0.32	2.692	0.906		
	AC	-17.25	-8.62	2.692	0.005		
	AD	-2.79	-1.39	2.692	0.611		
	AE	1.03	0.51	2.692	0.850		
	BC	10.88	5.44	2.692	0.058		
	BC	9.54	4.77	2.692	0.092		
	BD	5.93	2.96	2.692	0.285		
	BE	0.02	0.01	2.692	0.997		
	ABC	24.81	12.41	2.692	0.000		
	ABD	14.05	7.03	2.692	0.017		
	ABE	-20.82	-10.41	2.692	0.001		
	Ct Pt		4.96	8.076	0.782		

Regarding the results of *S. aureus* sterilization using PDBD, as shown in Table 5, when the results of the experiment were calculated by using the Mini-tab 16.1 program, it was found that all of the factors used in the experiment were significant, and that the R-squared value was 81.05%, as shown in Table 6. This means that the equation derived from this experiment is appropriate for use in real situations. In addition, the residual graph was a normal distribution for *E. Coli and S. Aureus*, as shown in Figure 9-10. The equation of the experiment can be written as follows.

$$Y = -9.93A + 17.68B + 3.17C + 10.90D - 0.64E - 17.25AB + 24.81ABC + 14.05ABD - 20.82ABE \quad (4)$$

The response optimizer function can be used to find the conditions that yield the result of the Y experiment closest to 100%: A = 0.966440, B = 1, C = -0.164325, D = -1, and E = 0.185970.

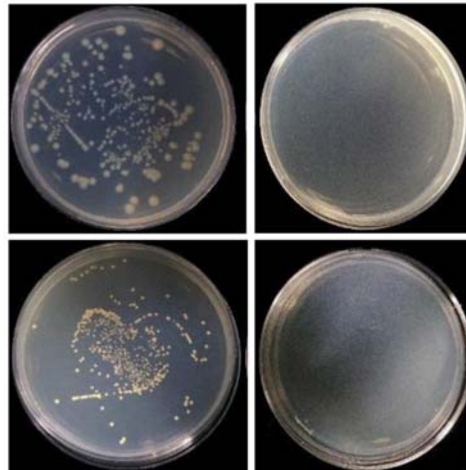


Figure 8. The PDBD sterilization test results between the initial *E. coli* on the scalpel handles (top left) with the sterilized scalpel handles (top right) and the initial *S. aureus* on the scalpel handles (bottom left) with the sterilized scalpel handles (bottom right).

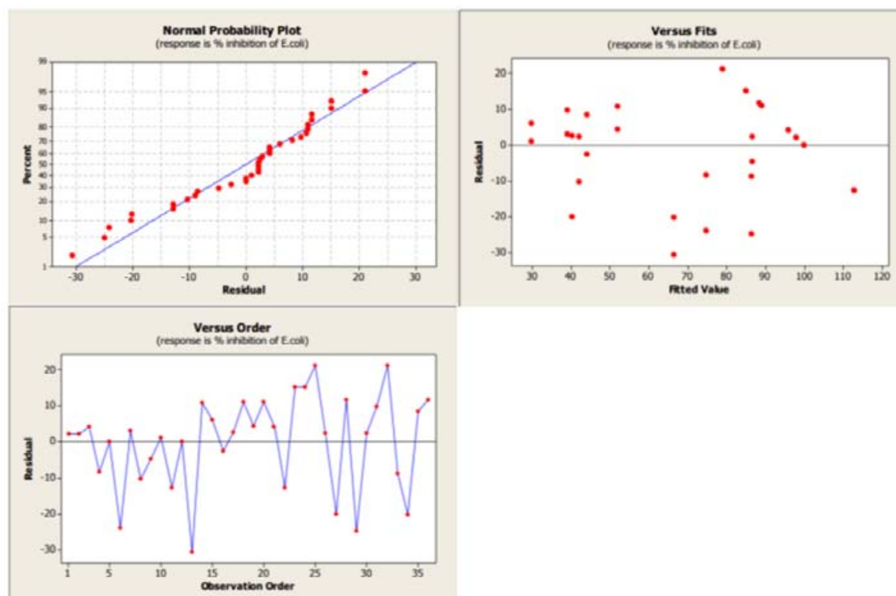


Figure 9. The distribution chart of the PDBD Mini-tab on *E. coli.*

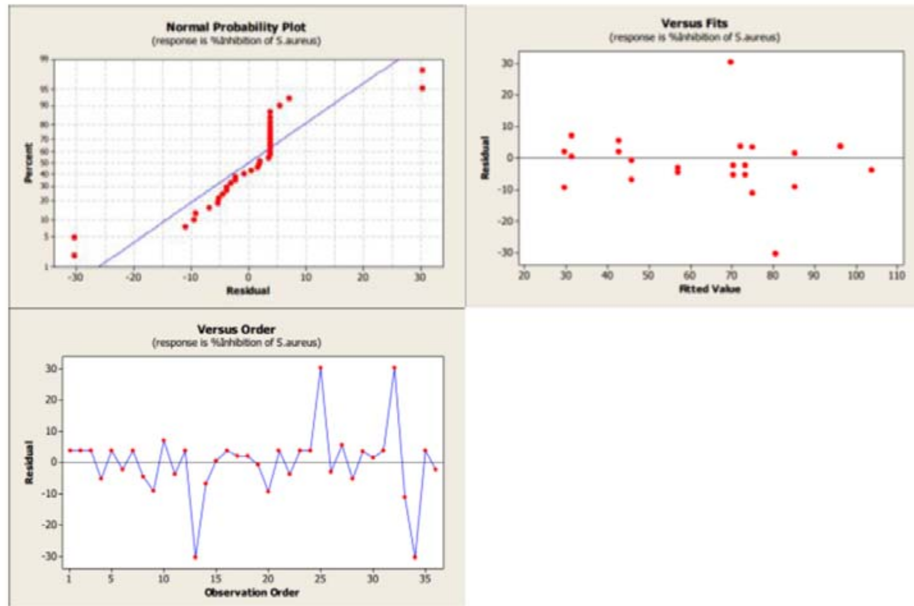


Figure 10. The distribution chart of the PDBD Mini-tab on *S. aureus*.

After using the selected criteria as the optimal and the effective parameters to perform 100% sterilization, when the scalpels were inspected after the plasma treatment to count the number of bacteria on the PDA plates, it was found that all the conditions were free from bacteria, or 100%sterilized, as shown in Figure 8.

3.3 OES Results of PS and PDBD

Regarding measurement of the OES values of the PS of sodium sulfate, sodium acetate, and PDBD under the best conditions, the results of the experimental design reveal that sterilization for *E. coli* and *S. aureus* had taken place. By determing the peak of the hydroxyl free radicals at 309 nm wavelength, PDBD could measure the light intensity to be 13,304 cps, while the PS light intensity was 250.177 cps and the light intensity of the PS of sodium acetate was 366.639 cps. Therefore, evidently, PDBD could produce the maximum number of hydroxyl radicals, as shown in the comparative graph, Figure 8.

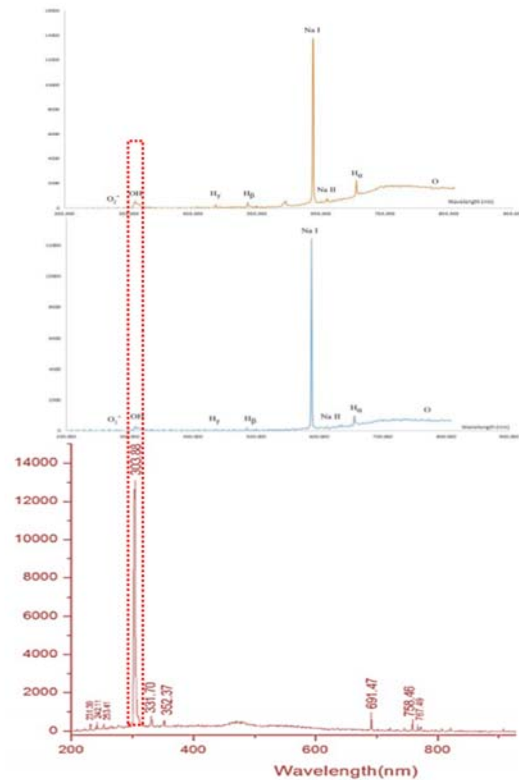


Figure 8. The OES test results of the PS of sodium sulfate, sodium acetate, and PDBD.

3.4 Differential of Thermal Effect between PS and PDBD

The PS and the PDBD sterilization techniques showed that both PS and PDBD are effective in eliminating *E. coli* and *S. aureus* bacteria. However, if it is taken into consideration as to how microorganisms are eliminated, it has been found that in PSs, the removal of microorganisms can occur due to many factors, such as heat from the working temperature resulting from the solution with better conductivity than the conductivity of air in the PDBD. Table 7 shows the relationship of power consumption with temperature when the temperature rises to almost 60 °C. For comparison, when the argon-based PDBD created plasma, on measuring the temperature, it was found that the temperature had increased slightly to 40 °C, the temperature at which plasma was produced in the PS. The gas exchange mechanism occurs before plasma.

Table 7. Concentration of solution, time, voltage, current, power, and temperature measured by experiment.

Solution	Concentration (g/700 ml)	Time (s)	V_{DC} (V)	I_{DC} (a)	P (w)	Temperature (°C)
Sodium Sulfate	1	20	520	0.2	104	48.5
		60	520	0.1	52	54.1
	2	20	600	0.2	120	53.2
		60	600	0.1	60	58.2
Sodium Acetate	1	20	520	0.2	104	46.9
		60	520	0.1	52	51.6
	2	20	600	0.2	120	51.4
		60	600	0.1	60	53.8

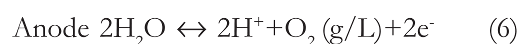
4. Discussion

Plasma activated in water technique is very effective for sterilized the microorganisms and this research used Plasma solution process to dissolve reactive species groups in the liquid with low voltage system. However It cannot generated hydroxyl group as much as our experiment design wanted but enough to killing bacteria for sterilized condition. The results of both the PS experiments, that is, experiments using sodium sulfate and sodium acetate solutions, showed that they were effective in sterilization, and that they could eliminate 100% of the *E. coli* and the *S. aureus* bacteria under different parameter conditions. With regard to the sodium acetate solution, it could eliminate the bacteria in more economical conditions because it used less time than the sodium sulfate solution which needed to take at least 40 s to sterilize 100%, while the sodium acetate solution used only 20 s. Both the solutions had equal voltages and concentrations: 520 V and a concentration of 1 g/0.7 L of water. The results are consistent with the results of the OES test, which found that hydroxyl free radicals from the PS of sodium acetate were reactive species with higher levels of efficiency, light intensity, and productivity of hydroxyl radicals than PS. The light intensity of the PS of sodium sulfate was 250.177 cps, while that of the PS of sodium acetate was 366.639 cps. Kozakova describes the mechanism of hydroxyl free radical sterilization [7]. The free radicals of hydroxyl pull the hydrogen atoms out of the microorganism's cells. It inhibits the growth

of the microorganism by inducing the peroxidation of the lipid, which is the element in the membrane layer, resulting in the loss of the osmotic ability of the membrane of the various substances. It also results in an oxidizing effect within the cell upon destruction of the molecular structure of the protein within the microorganism's cells [5]. The results are consistent with Takai which used the PS of sodium sulfate and sodium acetate for the elimination of *E. coli*. The type of power supply was a high-voltage pulsed type producing great numbers of hydroxyl radicals that were adequate for ionization in water [4]. Yong found that plasma ionization requires a voltage of at least 30 kV at a distance of only 1 cm between the electrodes to generate hydroxyl radicals efficiently and to have enough volume for sterilization [5]. However, when using a low voltage with the different sources of power supply in our experiment: DC power supply to generate plasma, an electrolyte solution is needed to increase the conductivity of the electricity to make the electrons run more efficiently but it cannot give as many as hydroxyl radicals as we expected. The light generated by the plasma generator is orange, which is the color of Na I. In comparison with PDBD sterilization, it can produce the same efficiency as that of PS, with 100% removal of *E. coli* and *S. aureus* at longer periods of time: the distance between the anode and the scalpel handles was 9.4 mm, 14 min, 76 watts, of argon, and a gas flow rate of 22 L per min for the *E. coli* bacteria; and the distance between the anode and the scalpel handles was 3.8 mm, 10 min, 66 watts, of argon, and a gas flow rate of 26 L per min for *S. aureus*. Therefore, when considering conditions that can eliminate both *E. coli* and *S. aureus* with 100% removal of the bacteria, ICP-OES results showed hydroxyl light intensity of 13,304 cps. The results are in line with Liu and Korachi who found that PDBD could produce hydroxyl free radicals with a remarkable 100% efficiency in removing *E. coli* [10, 12].

On providing voltage in the solution system, the gas is exchanged according to the process of reduction and oxidation within the electrolyte solution between the anodes and the cathodes, causing hydrogen microbubbles and oxygen from the water to become accumulated at the anodes and the cathodes, as shown in Equation 5 and Equation 6 [5].

Cathode $2H_2O + 2e^- \leftrightarrow 2OH^- + H_2$ (g/L) (5)



Anode $2H_2O \leftrightarrow 2H^+ + O_2$ (g/L)$+2e^-$ (6)

When the voltage is increased, the number of free radicals of the PS hydroxyl slightly increases, as shown in Figure 7, resulting in a light measured by Na I, which replaces the ionization that is the original goal, water ionization that produces hydroxyl radicals. When compared to PDBD, the difference in peaks is clearly visible at the wavelength of 309 nm.

5. Conclusion

Regarding the sterilization of both types of PS, sodium sulfate and sodium acetate, and PDBD, *E. coli* and *S. aureus* were 100% sterilized. However, sterilization using PDBD can produce a higher number of hydroxyl radicals than PS; however, PS is more convenient to apply, and can be used to sterilize materials having more complex forms, but it cannot be used to sterilize work that does not need moisture. Therefore, both PS and PDBD are appropriate for use in sterilization.

References

[1] Chu N.S., Chan-Myers H., Ghazanfari N. and Antonoplos P., *Am. J. Infect. Control*, 1999; **27(4)**: 315-319.

[2] Alfa M.J., DeGagne P., Olson N. and Puchalski T., *Infect. Control Hosp. Epidemiol.*, 1995; **17(2)**: 92-100. DOI 10.1086/647252.

[3] Saito Y., Kobayashi H., Uetera Y., Yasuhara H., Kajiura T. and Okubo T., *Am. J. Infect. Control*, 2014; **42(1)**: 43-47. DOI 10.1016/j.ajic.2013.06.022.

[4] Takai O., *Pure Appl. Chem.*, 2008; **80(9)**: 2003-2011. DOI 10.1351/pac200880092003.

[5] Yong Y., Yong I.C. and Alexander F., *Plasma Discharge in Liquid*, CRC Press Taylor & Francis Group 6000 Broken Sound Parkway NW, Suite 300, Boca Raton 10017, 2012.

[6] Zhang Q., Ma R., Tian Y., Su B., Wang K., Yu S. and Fang J., *Environ. Sci. Technol.*, 2016; **50(6)**: 3184-3192. DOI 10.1021/acs.est.5b05108.

[7] Kozakova Z., *Electric Discharges in Water Solutions*, PhD Thesis, Brno University of Technology, 2011.

[8] Moisan M. and Wertheimer M.R., *Surf. Coat. Technol.*, 1993; **59(1-3)**: 1-13. DOI 10.1016/0257-8972(93)90047-R.

[9] Feichtinger J., Schulz A., Walker M. and Schumacher U., *Surf. Coat. Technol.*, 2003; **174-175(0)**: 564-569. DOI 10.1016/S0257-8972(03)00404-3.

[10] Liu H., Chen J., Yang L. and Zhou Y., *Appl. Surf. Sci.*, 2008; **254(6)**: 1815-1821. DOI 10.1016/j.apsusc.2007.07.152.

[11] Schneider P.M., *Am. J. Infect. Control*, 2013; **41(5)**: 81-86. DOI 10.1016/j.ajic.2012.12.007.

[12] Korachi M., Gurol C. and Aslan N., *J. Electrostat.*, 2010; **68(6)**: 508-512. DOI 10.1016/j.elstat.2010.06.014.

[13] Schneider J., Baumgartner K.M., Feichtinger J., Kruger J., Muranyi P. and Schulz A., *Surf. Coat. Technol.*, 2005; **200(1-4)**: 962-966. DOI 10.1016/j.surfcoat.2005.01.114.

[14] Justan I., Cernohorska L., Dvorak Z. and Slavicek P., *Folia Microbiologica*, 2014; 1-6. DOI 10.1007/s12223-014-0301-y.

[15] Mrad O., Saloum S. and Al-Mariri A., *Vacuum*, 2013; **88(0)**: 11-16. DOI 10.1016/j.vacuum.2012.08.002.

[16] Yoshino K., Matsumoto H., Iwasaki T., Kinoshita S., Noda K. and Iwamori S., *Vacuum*, 2013; **93(0)**: 84-89. DOI 10.1016/j.vacuum.2013.01.009.

[17] Gates F.L., *J. Exp. Med.*, 1920; **31(1)**: 105-114.