Combination model of electric field and light for deactivation biofilm bacteria

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ABSTRACT. *The basic ingredients for making medical devices are varied, so they require different sterilization techniques. Sterilization techniques that do not cause heat are needed because not all medical devices are made from heat-resistant materials. This study aims to develop a mathematical model of deactivation of biofilm-forming bacteria with a combination of electric fields and light. Mathematical models are used to explain the mechanism of the decrease in the number of bacteria on biofilms. The mathematical model testing was only carried out at the electric field intensity of 2.5 - 4.0 kV / cm and the light intensity of 50 - 250 mW / cm^2 and in the biofilm of the bacterium Pseudomonas aeruginosa. The pulse duration of the electric field used is 50 µs, while the wavelength of light is 405 nm. Biofilm originated from the bacterium Pseudomonas aeruginosa grown on a catheter and incubated for 6 days at 37oC. Biofilm exposure was carried out at room temperature 30oC and environmental air humidity around 75%. The results showed that an increase in the electric fields and light caused an increase in the decrease in the number of bacteria. Decreasing the number of bacterial colonies that occur fulfills logarithmic functions. The decrease in the number of bacteria is caused by an increase in the amount of diffusion of water and ions that pass through the cell membrane, thereby damaging the cell membrane. Increased diffusion of water and ions that pass through the membrane occur because of the modulation of the external electric field with the electric field of the charge space produced by light. The electric field of space charge does not affect the occurrence of irreversible electroporation.*

RÉSUMÉ. *Les ingrédients de base pour la fabrication de dispositifs médicaux sont variés et nécessitent donc différentes techniques de stérilisation. Des techniques de stérilisation non génératrices de chaleur sont nécessaires car tous les dispositifs médicaux ne sont pas fabriqués en matériaux résistant à la chaleur. Cette étude vise à développer un modèle mathématique utilisant une combinaison de champ électrique et de lumière pour désactiver les bactéries formant des biofilms. Des modèles mathématiques sont utilisés pour expliquer le mécanisme par lequel le nombre de bactéries sur le biofilm est réduit. Le test du modèle mathématique a été réalisé uniquement dans une intensité de champ électrique de 2,5 à 4,0 kV / cm et une*

intensité lumineuse de 50 à 250 mW / cm^2 et dans le biofilm de la bactérie Pseudomonas aeruginosa. La durée d'impulsion du champ électrique utilisé est de 50 µs et la longueur d'onde de la lumière est de 405 nm. Le biofilm provevant de la bactérie Pseudomonas aeruginosa a été développé sur un cathéter et incubé pendant 6 jours à 37° C. Le biofilm est exposé à une température ambiante de 30 ° C et une humidité de l'air ambiante d'environ 75%. Les résultats ont montré qu'une augmentation des champs électriques et de la lumière entraînait un renforcement de la diminution du nombre de bactéries. La diminution du nombre de colonies bactériennes peut remplir des fonctions logarithmiques. La diminution du nombre de bactéries est provoquée par une augmentation de la quantité d'eau et d'ions diffusés qui traversent la membrane cellulaire, endommageant ainsi la membrane cellulaire. En raison de la modulation du champ électrique externe avec le champ électrique de l'espace de charge généré par la lumière, la diffusion d'eau et d'ions traversant la membrane augmente. Le champ électrique de la charge d'espace n'affecte pas l'occurrence d'une électroporation irréversible.

KEYWORDS: *biofilm, bacteria, field, electricity, light, combination, intensity, exposure*.

MOTS-CLÉS: *biofilm, bactéries, champ, électricité, lumière, combinaison, intensité, exposition*.

DOI:10.3166/ I2M.17.153-165 © 2018 Lavoisier

1. Introduction

Installation of implants especially breast implants often causes infections caused by bacteria (Monfort *et al*., 2012) which may be caused by medical devices or other. In medical devices, bacteria generally form biofilms. Bacteria that have formed biofilms are very resistant to several types of disinfectants, antibiotics, and chemicals (Neut *et al*., 2005), so it is very difficult to inhibit the growth. Sterilization uses heating (Gilbert *et al*., 2001) and electromagnetic wave radiation (Cheng *et al*., 2009) can cause damage to medical equipment due to heating. Therefore it is urgent to look for other sterilization techniques that do not cause heat. Sterilization using pulsed electric fields with an intensity of 38.4kV / cm has been shown to not cause heat in the sterilized material. Sterilization using pulsed electric fields has been shown to reduce the number of infecting bacterial colonies (Braxton *et al*., 2005; Montgomery *et al*., 2015; Chen *et al*., 2014). Therefore sterilization by combining electric fields and light is possible using an electric field with moderate intensity.

The electric field when interacting with bacteria causes electroporation of the cell membrane so that the cell membrane permeability is increased. Irreversible electroporation is achieved when the transmembrane potential exceeds the cell membrane threshold potential. In order for the irreversible condition to be achieved, the previous study used an electric field above 18 kV / cm with a duration of pulses of less than 2 µs. The high intensity of the electric field needed makes the cost of procuring equipment more expensive, making it difficult to meet the needs of lower-class health services.

Low-intensity light radiation in bacteria results in a photochemical reaction in bacteria (Christensen *et al*., 2007) and the reaction produces hydroxyl (OH) radicals and O_2^- superoxide anions (Lazar and Chifiriuc, 2010). OH and O_2^- are highly reactive when in contact with organic compounds, causing bacteria to die. Ultraviolet light

radiation on biofilms from *Escherichia coli* bacteria for 48 hours reduced the number of bacterial colonies by 1.1-3.8 logs, making it less effective. The interaction of light with biofilms also causes the absorption of photon energy by biofilms. Photon absorption by biofilms has the opportunity to generate electric fields in the charge space in the biofilm when excited electrons occur recombination. But the electric field of space charge that has been generated has not been able to make irreversible electroporation.

This research was conducted by combining electric fields and light with the aim of studying the mechanism of the decrease in the number of bacteria on biofilms. This technique is possible to reduce the intensity of the electric field needed to achieve irreversible electroporation on the cell membrane. This study uses a mathematical description derived from physics concepts to reveal the mechanism of decreasing the number of biofilm-forming bacteria. The modeling equations obtained are tested graphically and compared with graphs of empirical equations. Tests carried out by biofilm exposure using an electric field with an intensity of 2.5 - 4.0 kV / cm and light with an intensity of 50-250 mW / cm^2.

2. Experimental

2.1. Theory

At the atomic level, the electric field subject to material will cause a redistribution of the charge bond. Materials with less symmetry or not centrosymmetric centers will show electro-optical linear (Pockels) effects when in an electric field. At light wavelengths that correspond to optimum absorption, bacteria will be photoconductive (Jose *et al*., 2009). Photoconductive properties can give rise to a space charge field which in turn modifies the refractive index or is photorefractive. Exposure to a combination of electric and light fields causes an electric field modulation which can be expressed as

$$\boldsymbol{E}(x) = E_o - \frac{k_b T}{e}\frac{1}{I}\frac{\partial I}{\partial x} \tag{1}$$

Where *E(x)* is the electric field of space charge, E_o is the external electric field, k_b is the Boltzman constant, *e* is the electron charge, ∂x is the change in thickness, and ∂I is the change in light intensity. If the light used meets the equation $I(x) = I_o e^{-\alpha x}$, then

$$\boldsymbol{E}(x) = \left(\boldsymbol{E_o} - \frac{1}{x}\frac{k_b T}{e} ln\left({}^{I(x)}/_{I_o}\right)\right) \tag{2}$$

I(x) is the light intensity at *x* and α depth is the coefficient of absorption of bacteria. The existence of an electric field of space charge in the bacterial cell membrane causes a change in the refractive index expressed which is expressed by the equation

$$\Delta n = -\frac{1}{2} r n^3 \left(\boldsymbol{E_o} - \frac{1}{x} \frac{k_b T}{e} ln \left({}^{I(x)}/_{I_o} \right) \right) \quad (3)$$

Changes in the refractive index cause an increase in permeability to build bacterial cells, resulting in an increase in the dufusion of water and ions passing through the cell membrane. The next impact is the increase in the conductivity of the cell membrane. As a result of the increased conductivity of cell membrane, there will be damage to the cell membrane which causes bacterial death. The decrease in the number of bacteria that occurs can be approached by modifying the Weibull distribution equation, namely

$$\log \frac{N(t)}{N_o} = -\left(\frac{\sigma \left(1 + \frac{\Delta n}{n - n_a} \right) E(x)^2 t}{\delta} \right)^{\rho} \quad (4)$$

Where ρ is two Weibull probability density parameters, σ is the conductivity of the cell membrane. Sequential *N(t)* and N_o are the number of bacteria before and after treatment, while the Weibull probability density parameter δ is determined using the equation model based on the Gompertz function[0]

$$\delta = a - be^{e^{c(K-d)}} \quad (5)$$

Where δ is the dimension of energy required for the first inactivation, *K* is the electric field strength of the space charge, *a*, *b* and *c* are constants and *d* is the model parameter.

2.2. Research method

In the study of pulsed electric fields generated from a high voltage power supply connected to the switch, the next is connected to parallel chips of titanium. The specification of high voltage power supply is output voltage 0 to ± 10 kV DC, output current output is 0 to ± 20 mA DC. The switch used has an oscillation frequency range of 0 Hz - 100 kHz. The light used to expose is a Laser Diode that operates at a wavelength of 405 nm with a maximum output power of 500 mW and is a continuous wave. The equipment is arranged as shown in Figure 1. The sample in this study is the *Pseudomonas aeruginosa* bacteria that has formed Biofilm. Biofilms were grown on a catheter for 6 days. Exposure is done by a combination of electric and light fields. The electric field strength is 2.5-4.0 kV / cm with a pulse duration of 50 μs and light intensity of 50-250 mW/cm^2. Data were analyzed graphically and descriptively and compared with the results of modeling equation 4.

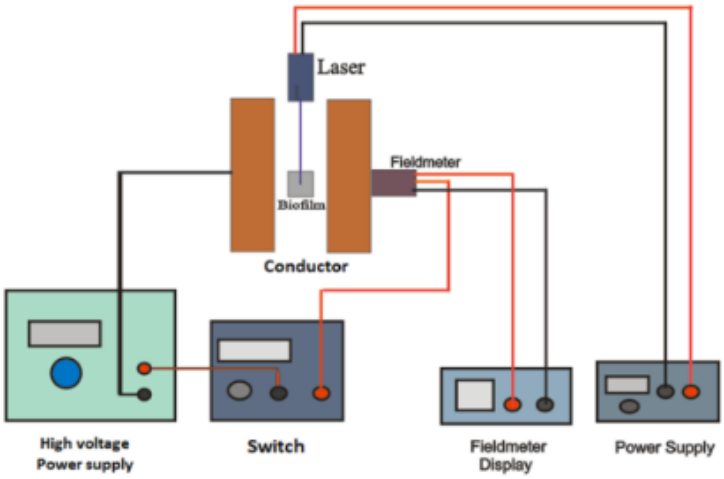

Figure 1. A scheme of research tools

3. Results and discussions

3.1. Results

Exposure to electric field strength 2.5 - 4.0 kV/cm with a light intensity of 50 mW/cm^2 for 5 minutes obtained a decrease in the number of bacteria such as Table 1. Calculation of the decrease in the number of bacteria is done using the equation

$$\text{Decrease} = \log (N_t/N_0) \quad (6)$$

Where N_t is the number of bacteria after being exposed for t seconds and N_o is the number of bacteria before being exposed. The data showed that exposure to an electric field strength of 4.00 kV/cm log resulted in a decrease in the number of bacteria by -2.68 log cfu/ml. Graphically the decrease in the number of bacteria can be seen as Figure 2. If it is expressed by a mathematical function graph in Figure 2. Meet the equation

$$y = -0{,}03953\, e^{-\frac{x}{0{,}99755}} - 0{,}42353 \quad (7)$$

With the coefficient of determination (R^2) equal to 0.95906. Where *y* reveals a decrease in the number of bacteria, while *x* reveals electric field strength. The graph of the data ploting when compared with the graph of the modeling results looks like Figure 3. On the electric field strength 3.0 - 3.75 kV/cm the decrease in the number of bacteria obtained from the modeling graph is lower than the data, while in the electric field strength 4, 0 kV/cm is the opposite.

Table 1. Data of the decrease in the number of bacteria at 50 mW/cm^2 of light intensity and 5 minutes of exposure time (Pseudomonas aeruginosa)

No	Electric field (kV/cm)	Average number of bacteria (x 10^{10} cfu/ml)	Log Reduction (log cfu/ml)
1	control	43.37±3.10	0
2	2.50	7.070±0.13	-0.79
3	2.75	2.965±0.54	-1.17
4	3.00	2.566±0.29	-1.23
5	3.25	1.212±0.04	-1.55
6	3.50	0.880±0.07	-1.69
7	3.75	0.440±0.06	-1.99
8	4.00	0.092±0.00	-2.68

Exposure to light intensity from 0 - 250 mW/cm^2 with an electric field strength of 3.0 kV/cm with 10 minutes exposure time obtained data as shown in Table 2. Graphically the decrease in the number of bacteria that occurs is shown in Figure 4. Mathematically a decrease in the number of bacteria happens can be approximated by the equation

$$y = -0{,}36034\ e^{\frac{x}{463{,}30448}} - 2{,}27509 \quad (8)$$

with the coefficient of determination (R^2) equal to 0.9994. The graph of the results of the data when compared with the modeling results is shown in Figure 5. The graph shows that the modeling results are lower than the data ploting. However, the chart pattern that is formed is relatively the same.

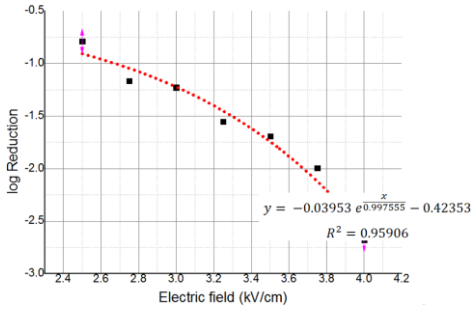

Figure 2. Graph of the effect of the electric field on the decrease in the number of Pseudomonas aeruginosa bacteria with a light intensity of 50 mW/cm^2 and a 5 minute exposure time

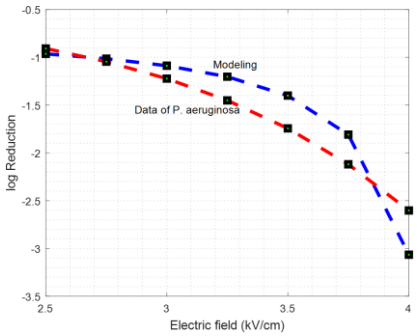

Figure 3. Graph of the effect of electric field strength on the decrease in the number of bacteria in light intensity of 50 mW/cm^2 and the exposure time of 5 minutes

Table 2. Data on the decrease in the number of bacteria at a field strength of 3.0 kV/cm and a 10-minute exposure period (Pseudomonas aeruginosa)

No	Light intensity (mW/cm^2)	Average Number of bacteria (x 10^8 cfu/ml)	Log Reduction (log cfu/ml)
1	controls	43.36±3.10	0.00
2	0	1.95±0.69	-1.355
3	50	1.72±0.22	-1.402
4	100	1.56±0.05	-1.444
5	150	1.39±0.13	-1.494
6	200	1.22±0.09	-1.551
7	250	1.05±0.06	-1.616

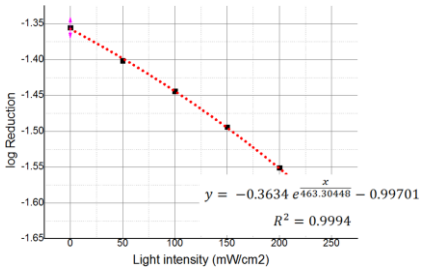

Figure 4. Graph of the effect of light intensity on the decrease in the number of Pseudomonas aeruginosa bacteria with an exposure time of 10 minutes and an electric field strength of 3.0 kV/cm

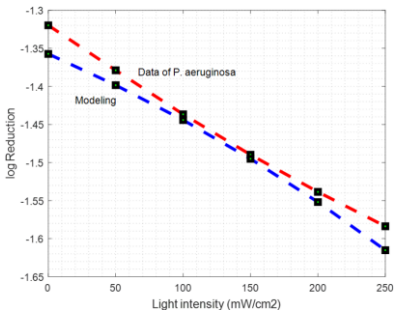

Figure 5. Graph of modeling results of the effect of light intensity on the reduction in the number of bacteria assuming an electric field strength of 3.0 kV/cm (Pseudomonas aeruginosa)

3.2. Discussion

Exposure to a combination of electric and light fields in biofilm making bacteria is illustrated as Figure 6. The circuit consists of biofilm, electrical potential, and light. V_T electric potential is connected to resistor *R* and two electrodes with the distance between pieces is d. The potential difference in the resistor is V_R and at the electrode is *V*. Thus the electric field strength E generated at the electrode satisfies the equation E = V/d. For example J is the current density in the circuit and A is the surface area of the electrode, then *VR = JAR*. Because $V_T = V + V_R$, then

$$E_T = \frac{1}{d}(V_T - JAR) \tag{9}$$

For example, light is observed in the biofilm in the direction of the optical axis z and is only allowed to refract in the direction of *z*. The optical axis leads to the c-axis oriented along the *x* coordinate. Due to the external electric field, the light is polarized linearly towards the x axis. In this condition the extradinory refractive index ne along the c axis is given by equation ($n(E) = n - 1/2\ rn^3\ E_{SC}$) where *r* is the electro-optical coefficient, and E_{SC} is the space charge induction[0].

As a result of the light exposure in the *z* direction, absorption of photons by the cell membrane of the biofilm constituent bacteria occurs and causes the transfer of electrons from the valence band to the conduction band[0]. The average photoexcitation $(G\bar{)}$ which occurs in magnitude is proportional to the light intensity and electron density in the unexplained valence band. Exposure to using non-uniform intensity of light causes differences in excited electron density, resulting in charge diffusion. In addition to diffusion there is also a driff current due to the presence of an electric field, and allows current to occur from the photovoltaic effect. The density of the drift, diffusion and photovoltaic effects of the magnitude of the magnitude of the position, so that the current density is expressed as (Puértolas *et al*., 2009).

$$J = e\,\mu\eta E_{SC} - k_b T\mu\frac{d\eta}{dx} + k_p S_i(N_V - N_V{}^+)I \tag{10}$$

$$\frac{\partial J}{\partial x} = 0 \text{ or } J = \text{constant} \tag{11}$$

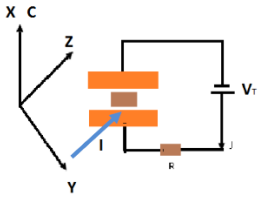

Figure 6. Illustration of a series of biofilm-making bacteria, electric fields, and laser light

The diffusing electrons allow recombination *(R)* at another location whose magnitude is proportional to the amount of density ɲ, and the number of electron densities of ionized valence bands (traps) $N_V{}^+$. Recombination trapped in other locations, causing negative charges, while the location left behind becomes positively charged, resulting in an electric field of space charge. The electric field of the E_{SC} space charge generated depends on the position, which is expressed as.

$$\frac{\partial \boldsymbol{E_{sc}}}{\partial x} = \frac{e}{\varepsilon_o \varepsilon}(N_V{}^+ - N_K - ɲ) \tag{12}$$

Where $N_V{}^+$ and N_V are respectively the density of the ionized valence band and the density of the valence band. N_K is the conduction band density, is the electron density, S is the photoexitation section, the average recombination of the charge carrier, μ and e are the mobility and electron charge, k_p is the photovoltaic constant, k_b is the Boltzmann constant, T is the absolute temperature, ε_o is permittivity vacuum, I_d is dark radiation intensity, $I = I\,(x, z)$ is the profile of light power density.

Simplifying the decline, it is observed that the biofilm compiler bacteria are considered to be truly photovoltaic and photorefractive, so that they apply $N_v >> ɲ$, $N_v{}^+ >> ɲ$, and $N_k >> ɲ$. If the light density of the light beam is changed slowly with respect to the direction of x, then the peculiarities of the photovoltaic-photorefractive media are dimension $\left|\left({}^{\varepsilon_o \varepsilon_r}/_{eN_K}\right)\left(\frac{\partial E_{SC}}{\partial x}\right)\right|$ is lower than one. In this situation the space load field can be determined in an approach and obtained

$$E(x) = gE_a \frac{I_x + I_d}{I + I_d} + E_p \frac{gI_x - I}{I + I_d} - \frac{k_b T}{e} \frac{1}{I + I_d} \frac{\partial I}{\partial x} \tag{13}$$

With $g = \frac{1}{1 + pSR(I_x + I_d)}$

It is generally $0 \leq g \leq 1$ which implies that only the bias part of E_a can be applied to the media. By removing R or $R = 0$ then $g = 1$, $E = V_T/d = E_o$, thus

$$\boldsymbol{E}(x) = E_o \frac{I_x + I_d}{I + I_d} + E_p \frac{I_x - I}{I + I_d} - \frac{k_b T}{e} \frac{1}{I + I_d} \frac{\partial I}{\partial x} \tag{14}$$

In a narrow area, $I \sim I_x$, while $I_d = 0$. Thus equation 14 becomes

$$\boldsymbol{E}(x) = \boldsymbol{E}_o - \frac{k_b T}{e}\frac{1}{I}\frac{\partial I}{\partial x} \tag{15}$$

Equation 15 shows that the combination of electric and light fields in this case is one form of modulation. If the light used meets the equation, then it is obtained

$$\boldsymbol{E}(x) = \left(\boldsymbol{E_o} - \frac{1}{z}\frac{k_b T}{e} ln\left({}^{I(z)}/_{I_o}\right)\right) \tag{16}$$

when $k_T = \frac{k_b T}{ze}$, then

$$\boldsymbol{E}(x) = \left(\boldsymbol{E_o} - k_T ln\left({}^{I(z)}/_{I_o}\right)\right) \tag{17}$$

Thus changes in the refractive index can be expressed as

$$\Delta n = -\frac{1}{2} r n^3 \left(\boldsymbol{E_o} - k_T ln\left({}^{I(z)}/_{I_o}\right)\right) \tag{18}$$

Exposure to a combination of electric and light fields in biofilm constituent bacteria will change the refractive index like equation 18. Changes in the refractive index trigger changes in cell membrane permeability and can be rationalized by diffusion of water and ionic environment into the lipid group interface region. The relationship between the refractive index *n(I, E)* with the diffusion of water and ions into the cell membrane is expressed as:

$$n(I, E) = (1 - \Delta f_W) n + \Delta f_W n_a \tag{19}$$

So that

$$\Delta f_W = \frac{\frac{1}{2} r n^3 \left(E_o - k_T ln\left({}^{I(z)}/_{I_o}\right)\right)}{n - n_a} \tag{20}$$

By taking a definition of $k_n = \frac{r n^3}{n - n_a}$, it can be simplified into

$$\Delta f_W = \frac{1}{2} k_n \left(E_o - k_T ln\left({}^{I(z)}/_{I_o}\right)\right) \tag{21}$$

Increased permeability triggers changes in the diffusion of water and ions that enter the cytoplasm. Therefore, the higher the membrane permeability, the lower the number of cells that can survive. If the threshold conditions for permeability have been reached, then the number of bacterial deaths is not linear with the light intensity and electric field strength used.

Changes in the diffusion of water and ions passing through the bacterial cell membrane cause an increase in electrical conductivity. The amount of electrical conductivity can be determined by

$$\sigma(E) = \sigma(0)+\Delta\sigma \tag{22}$$

Substituting equation 21 into equation 22 is obtained

$$\sigma(E) = \sigma\left(1+\frac{1}{2}k_n\left(\boldsymbol{E_o} - k_T ln\left({}^{I(z)}/_{I_o}\right)\right)\right) \tag{23}$$

As a result of the increased conductivity of the cell membrane causing membrane damage, allowing bacteria to die. Reduction in the number of bacteria that occurs can be determined by

$$\log\frac{N(t)}{N_o} = -\left(\frac{W}{\delta}\right)^{\rho} \tag{24}$$

With [165]

$$W = \int \sigma(E)\boldsymbol{E}^2 dt \tag{25}$$

Substitution of equation 23 to equations 25 and 24 is obtained

$$\log\frac{N(t)}{N_o} = -\left(\frac{\sigma\left(1+\frac{1}{2}k_n\left(\boldsymbol{E_o}-k_T ln\left({}^{I(z)}/_{I_o}\right)\right)\right)\boldsymbol{E}(x)^2 t}{\delta}\right)^{\rho} \tag{26}$$

Equation 26 is a model for reducing the number of bacteria due to exposure to a combination of electric fields with light. Where *N(t)* is the number of bacteria after exposure, N_o. is the number of bacteria at first.

4. Conclusion

The mechanism of inhibition of the growth of biofilm constituent bacteria using exposure to a combination of electric and light fields is caused by electroporation of the cell membrane. Electroporation occurs allegedly due to modulation between the external electric field and the electric field of the space charge due to irradiation. This modulation electric field will reduce the refractive index of biofilm constituent bacteria, so that the permeability increases. Increased permeability of cell membranes causes increased diffusion of water and ions into the cell membrane. Increased diffusion of water and ions increases membrane conductivity and ultimately damages cell membranes.

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