Modeling the effect of Rose Bengal on growth and decay patterns of Pseudomonas aeruginosa, Escherichia coli and Staphylococcus aureus

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Abstract: Most infections caused by (Pseudomonas aeruginosa, Escherichia coli and Staphylococcus aureus) are hospital and community acquired infections in patients. Bacterial growths incorporated with photosensitizing material (Rose Bengal) with and without light were investigated. The results demonstrated that the viable counts are increasing in absence of light (in dark) for all samples incorporated with Rose Bengal. Variation in growth phases were noticed as expected, but there is no significant change in decay phases. Convenient and adequate mathematical modeling is in very good agreement with the experimental results and showed to be a very good approach of characterization the growth behaviors of the bacteria. Bandwidths are independent of bacteria group (gram-positive or gram-negative) but it seems totally dependent on the oxygen requirements; an anaerobic bacterium takes broader bandwidths than aerobic bacteria. This concludes that the growth and lethal rates of anaerobic are much greater than aerobic.

Keywords: Pseudomonas, E coli, Staphylococcus, growth curve modeling, Rose Bengal

1. Introduction

Several studies and mathematical modeling on bacterial growth phases which have been conducted by many researcher, lead to significant results in understanding the bacterial growth behaviors. In spite of significant achievement in understanding the bacterial behaviors, there is still no mathematical strong evidence about the correlation between the strain or type of bacteria and growth and/or mortality rates. Up to our knowledge, this is the first time Gaussian function is applied to express the bacterial behaviours in terms of bandwidths and type of bacteria. Three of most important bacteria named Pseudomonas aeruginosa, Staphylococcus aureus, and Escherichia coli are used in this work. In spite of the fact that they are common colonizers of human skin and mucous membranes, they are responsible for a wide spectrum of infections targeting a broad range of organs and deep-seated infections and surgical site infections like wound infections and endophthalmitis [1, 2].

Pseudomonas aeruginosa and Escherichia coli are Gram-negative and Staphylococcus aureus is a Gram-positive bacteria. Those types of Bacteria are considered to be the most virulent human pathogens which rarely cause infection in the healthy host. They are found in a variety of habitats including soil and fresh water streams as well as on plant and animal tissues [3, 4]. Members of this genus are characterized by their ability to grow in water because of their simple nutritional requirements: they will even grow in distilled water and natural mineral water [5].

Pseudomonas aeruginosa is of particular interest as a human pathogen which is adept at infecting
different tissues and organs. It is considered to be an opportunistic and human pathogen as it causes infections in individuals with neutropenia or whose normal immune status is compromised in some way. Although most infections caused by Pseudomonas aeruginosa are hospital acquired, community acquired infections in patients have also been reported [6, 7]. Epidemiological statistics indicate significant frequencies of occurrence of Pseudomonas aeruginosa infections and capable of transiently colonizing skin and mucosal surfaces, but deficient in its ability to carry out initial steps of infection [8]. The normal non-specific defenses of the human body are sufficient to prevent the bacteria from causing infections but once the opportunist, it could override the primary defense systems and may cause several fatal systemic diseases in the human body [9 - 13]. Mortality rates attributed to this bacterium in hospital acquired infections and patients suffering from cystic fibrosis are high [14]. The capacity of this organism to cause such high rates of illness and death is primarily due to a diverse range of virulence factors [15, 16]. Many outbreaks of food-borne illness are reported worldwide in which Staphylococcus spices were a common cause of food poisoning (enterocolitis). Some strains also release superantigens, which interact with the immune system causing the serious systemic disease, Toxic Shock Syndrome [17]. Staphylococcus aureus is a primary and major cause of nosocomial infections which is considered to be the second leading cause of nosocomial blood stream infections [18, 19]. Escherichia coli are commonly found in warm-blooded organisms and can cause serious food poisoning in humans and preventing the establishment of pathogenic bacteria within the intestine. Virulent strains of Escherichia coli can cause gastroenteritis, urinary tract infections, and neonatal meningitis. Virulent strains are also responsible for haemolytic-uremic syndrome, peritonitis, mastitis, septicemia [20]. Some other disturbance of cell-wall synthesis and the appearance of a multilamellar structure near the septum of dividing cells, along with loss of potassium ions from the cells were reported by [21].

In general, those bacteria remain a threat to public health with a high rate of morbidity and mortality. The goal of this study is to get better understanding and explain the behavior of the bacteria growth stages with and without additives in presence and absence of light. Those stages described by numerous mathematical modeling that varies considerably in structure and number of parameters. Therefore, accurate parameter of the growth curves such as the relationship between the bandwidth and bacteria group at half maximum value is still needed and up to our knowledge it has not been calculated.

Furthermore, investigation the effect of irradiation on the bacterial growth of different strains of bacteria namely Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli with the presence of Rose Bengal dye were studied.

2. Mathematical Modeling

Several sigmoidal functions such as (Logistic, Gompertz, and Richards) were compared to describe growth curve or lethal damage of several organisms [22 - 25].

A new calculation of the two main stages of bacterial growth and decay were fitted with Gaussian equation. In addition, a Logistic model for bacteria growth with and without additives were applied to experimental data obtained in this study. The new model successfully describes Gaussian and sigmoidal growth curve of Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus under various initial conditions.

In general, the growth curves of bacteria are typical sigmoidal curves [26]. Original logistic model can be expressed as:

\[ \frac{dN}{dt} = rN \left(1 - \frac{N}{N_{\text{max}}} \right) \] ........................ (1)

Where N is the population of the organism at time t and r is rate constant, or the maximum specific growth rate. \( N_{\text{max}} \) is the maximum population (at the stationary phase), the term \( (1 - \frac{N}{N_{\text{max}}} \) which suppresses the growth rate at a high population.

When N is small during the lag phase, the value of this term is almost one, which does not affect the growth rate. During the stationary phases, the values of \( N_{\text{max}} \) approaches zero with growth rate almost zero. Sigmoidal growth curves described in this study could be explained on the bases of Gibson et al.
modified logistic model to fit bacterial growth data by the following equation:

\[ N = \frac{a}{1 + \exp(-\alpha (t - \beta))} \] ........................ (2)

Where \( N \) is the number of colony, \( a \) is the maximum number of colonies (always normalized to one), \( \alpha \) is the growth or lethal rate, \( t \) is the bacterial growth time, and \( \beta \) is the time required to reduce the maximum number of colonies to one half of its maximum value.

This study is focused on a mathematical modeling for the bacterial growth and lethal curves behaviors due to the importance of such bacteria which has increased dramatically in the last two decades. On the other hand, the following Gaussian Equation was used to fit the entire stages of the bacterial growth curves:

\[ N = a e^{-(t-b)^2 / 2c^2} \] ........................ (3)

where \( a \) is the maximum height of the curve peak, \( b \) is the position of the center of the peak, and \( c \) is the full width at the half maximum of the peak.

3. Materials and Methods

3.1. Materials

The following materials were used in this study are Rose Bengal (received from NEWTECH LTD, Brixworth, Northants. UK), Muller Hinton Agar (received from HiMedia Laboratories Pvt. Ltd, India) and Muller Hinton broth (received from HiMedia Laboratories Pvt. Ltd, India).

The broad-band light source used for bacteriolysis measurements was a 200-W high-pressure Hg/Xe arc. The lamp was housed in an Oriel Research Arc Lamp Housings model 66903, with the Oriel Digital Arc Lamp Power Supply Model 68907. For irradiation of the samples, the light source was generated from a 1.5 inch Oriel Research Arc Lamp Housings, (Copyright © 1996-2008 Newport Corporation) with an output power of (50-500 W) and light output intensity of 200 watts. The sample was located at 17 cm from the light source and the output intensity reach the samples were about 60J/cm² as measured by FiledMaxII Laser power/Energy Meter/Coherent/USA. Appropriate spot size to cover the sample area was created with an objective lens.

3.2. The Growth Characteristics

In order to estimate the rate of microbial reproduction, it is necessary to determine the numbers of microorganisms present. The growth of bacteria can be estimated by several different methods, including the total count, the viable count and turbidometric procedures. The viable plate count method is one of the most common procedures for the enumeration of bacteria. It is used to determine the viable population in a bacterial culture. In this procedure, a series of dilutions of a bacterial suspension are plated onto a suitable solid growth medium in order to determine the number of colonies. It is assumed that each colony arises from an individual bacterial cell. Therefore, by counting the number of colonies that develop and by taking into account the dilution factors, the concentration of bacteria in the original sample can be determined as Colony-Forming Units (CFU). A major limitation of the viable plate count procedure is its selectivity. It measures only those cells that are capable of growth on the given plating medium under the set of incubation conditions that are used. Sometimes cells are viable, but nonculturable unless steps are taken to acclimate the microorganisms to laboratory culture conditions. Bacteria can also be enumerated by direct counting procedures (total count or microscopy), that is, counting without the need to first grows the cells in culture. However, the difficulty in establishing the metabolic status of the observed bacteria, that is, whether the cells are living or dead, is a major limitation of this procedure.

3.3. Experimental Approach

The overnight culture of Pseudomonas aeruginosa ATCC 27853, Escherichia coli ATCC 25922, and
Staphylococcus aureus ATCC 29213 were performed as follows:
25 mL of a sterile medium Muller Hinton Broth (MHB) in a 100 mL Erlenmeyer flask were inoculated with a pure culture of each bacteria (single colony, from a streak plate). The flasks were then incubated in total darkness overnight in an incubator at 37°C. For the test culture of each bacteria a fresh aliquot (75 mL) of medium (MHB) was inoculated with 250 μL of each bacteria mentioned above for overnight culture. This was performed in duplicate (flasks A & B). The flasks were incubated at 37°C in the incubator.

For Preparing Culturing Media: 38 grams of Muller Hinton agar were suspended in 1000 ml of distilled water. The suspension was boiled and dissolved completely. The medium was sterilized by autoclaving at 15-lbs pressure and 121°C for 15 minutes with well mixing before use.
Similarly, 21 grams of Muller Hinton agar were suspended in 1000 ml of distilled water and heated to dissolve the medium completely dispense as desired and sterilize by autoclaving at 15-lbs pressure and 121°C for 15 minutes.

3.4. Viable Count
An aliquot (1 mL) of the identical test culture (flask B) was aseptically removed and serially diluted in the range of 10^-2 to 10^-9. Aliquots (100 μL) of the dilutions were spread plated onto appropriate agar plates Muller Hinton Agar (MHA). These plates were prepared in triplicate (three plates for each dilution). The plates were incubated overnight at 37°C. After 24 hours, colonies were counted in each plate and viable counts were calculated according to the following equation:

$$CFU/mL^-1 = N (1/DF) \text{ ........................................(4)}$$

Where; $CFU$ = The number of colony forming units, $N$ = Mean of counts on the plates and $DF$ = Dilution factor.

3.5. Sample Preparation
Fresh bacteria samples with seventeen hours old (Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli) were used throughout this study. Three samples from each kind of bacteria were prepared and kept inside the incubator. 100μl of bacteria was taken every one hour and cultured in a Petri dish for 24-hour long.

3.6. Bacteria Induced by Rose Bengal
Fresh bacteria samples with seventeen hours old (Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli) were used throughout this study.
Three different mixtures of bacteria (Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli) with drugs at different concentrations were prepared. Samples are mixed with photosensitizes drugs (Rose Bengal) and split every sample into two samples and irradiate the first one with light for continuous exposure times and the second one was incubated at total darkness. 100μl of bacteria were taken every 15 minutes and cultured in a Petri dish up to about 2 hours.
The concentrations range of the photosensitizing drug in the sample are (0.2mg/ml -0.6mg/ml). The samples were irradiated with 200-watt high pressure arc lamp.

4. Results
4.1 Samples with No Additives
The number of colonies in Petri dishes was measured and plotted for all the growth stages of three kinds of bacteria, Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli.

Figures 1, 2, 3 and Table 1 showed the relationship between the numbers of colonies versus growth time (0-26 hours).
The typical growth curves of Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa are
shown in Fig.1. The figure represents the living phases of the bacteria growth for Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa. The first phase represents the lag phase which takes about 3, 4, and 10 hours respectively. The second phase, which is called log (or exponential) phase, takes 11, 10, and 8 hours respectively. The third phase is the stationary phase and its duration is about 3 hours for the three kinds of bacteria. The fourth phase is called the death phase (decay) which takes 10, 10, 6 hours respectively.

![Figure 1](image_url)

**Figure 1:** A typical growth stages of Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa at temperature of (37° C) without any additives induced to the samples. The symbols denote the experimental values and lines represent the fitting of Gaussian Equation no. 3.
Figure 2: Growth curves for *Escherichia coli*, Staphylococcus aureus, and *Pseudomonas aeruginosa* samples without induced any additives. The symbols denote the experimental values and the lines represent the fitting function.
Figure 3: Lethal curves for Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa samples without induced any additives. The symbols denote to the experimental values and the lines represent the fitting function.

Table 1: The values of the model parameters ($a$, $β$, $α$, and $R^2$) represented in the modified logistic function and ($a$, $b$, $c$, and $R^2$) represented in Gaussian fit for the Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus aureus bacterial without additives at temperature of 37°C:

| Modified Logistic | Gaussian Fit |
|-------------------|--------------|
|                   | $a$          | $α$(hr$^{-1}$) | $β$(hr) | $R^2*$ | $a$          | $b$ | $c$ | $R^2*$ |
| E. coli Growth     | 1.07±0.033   | 0.49±0.03      | 8.04±0.20 | 0.995 | 1.03±0.01  | 14.08±0.08 | 5.12±0.08 | 0.996 |
| coli Lethal       | 1.04±0.032   | -0.52±0.04@    | 6.31±0.20 | 0.993 | 1.03±0.02  | 14.67±0.10 | 4.71±0.10 | 0.994 |
| S.a Growth        | 1.09±0.036   | 0.45±0.03      | 9.20±0.23 | 0.995 | 1.03±0.02  | 14.67±0.10 | 4.71±0.10 | 0.994 |
| Lethal            | 1.03±0.012   | -0.69±0.04@    | 5.40±0.10 | 0.997 | 1.03±0.02  | 14.67±0.10 | 4.71±0.10 | 0.994 |
| P.a Growth        | 1.11±0.02    | 0.67±0.03      | 15.23±0.10 | 0.998 | 1.06±0.01  | 18.94±0.05 | 3.29±0.05 | 0.998 |
| Lethal            | 1.13±0.10    | -0.70±0.12@    | 3.55±0.375 | 0.981 | 1.06±0.01  | 18.94±0.05 | 3.29±0.05 | 0.998 |

Results are expressed as means ± SD.
The values of the parameters were obtained by fitting the data to Eq. 2 and Eq. 3.
*Correlation Factor.
@ -Ve signs means the bacteria are in death phase (curves decaying down)

For simplicity and comparison, Gaussian fitting to those curves can be approximately considered as a two phases, growth and decay only. Growth curves of Escherichia coli and Staphylococcus aureus are almost behaving the same and it takes about 14 to 15 hrs to reach the maximum height while Pseudomonas aeruginosa takes longer time about 19 hours to reach the maximum height. Decay curves of Escherichia coli and Staphylococcus aureus are also behaving the same and it takes about 11.5 hours to reach the minimum values while Pseudomonas aeruginosa takes shorter time (about 7 hours) to reach the lowest value. Moreover, over all behaviors there is a lag time of about 4.5 hours in growth and decay phases as compared (Escherichia coli and Staphylococcus aureus) with Pseudomonas aeruginosa. Interestingly, there is a variation in growth phases in all types of bacteria, as expected, with no significant change in decay phases. The bandwidths at the half-maximum values of the average spectrum were calculated from the Gaussian fitting and found to be 12.9 hrs, 11.8, and 8.0 for Escherichia coli, Staphylococcus aureus,
and Pseudomonas aeruginosa, respectively. Interestingly, another approach of calculations was performed by fitting the exponential log phase (Figure 2) and death (decay) phase (Figure 3), separately. Growth parts, represent the number of colonies, increase with time (data points in Figure 2) and lethal parts, represent the number of colonies, decrease with time (data points in Figure 3). Data was normalized to one and fitted (solid lines) with logistic function Eqn. 2 using KaleidaGraph 4.0.

Figure 2 and Figure 3 represent the survival and death phases of the bacteria lifecycle without inducing any additives. Data points in the figures was fitted using the theoretical model Eqn.2, and the values of the model's parameters ($a$, $\alpha$, and $\beta$) for survival phase and death phase are summarized in Table 1.

Table 1 shows the parameters of Gaussian and logistic models. The last column of the table shows the correlation factor between experimental data points and theoretical model symbolized in solid lines. Comparing exponential phase with lethal phase, we can notice that the rate parameter $\alpha$(hr$^{-1}$), in case of growth phase, is lower than that of lethal phase, where the minus sign means the viable cell population declines. In the same patterns, the time $\beta$ required to reduce the maximum numbers of colonies to one half of its maximum value were found to be higher in case of growth phase as compared with the lethal phase. Fitting the growth curves of Pseudomonas aeruginosa with induced Rose Bengal at concentration of (0.2 – 0.6) mg/ml in the presence of light showed best fit with best values of parameters. Figure 4 represents the lethal phase of the Pseudomonas aeruginosa with induced Rose Bengal at concentrations of (0.2 - 0.6) mg/ml, respectively. The experimental curves were fitted using the logistic theoretical model and the values of parameters ($a$, $\alpha$, and $\beta$) are respectively shown in Table 2.

Table 2: The values of the variables ($a$, $\beta$, $\alpha$ and $R^2$) which represented in the logistic model for the irradiated (in light) samples of the Pseudomonas aeruginosa bacteria with induced Rose Bengal at different concentration from 0.2mg/ml to 0.6 mg/ml at temperature of 25°C:

| Concentration of Rose Bengal | Parameters               |
|-----------------------------|--------------------------|
|                             | $a$            | $\alpha$(hr$^{-1}$) | $\beta$(hr) | $R^2$  |
| 0.2mg/ml                    | 1.03±0.007     | 0.107±0.004          | 38.5±0.46    | 0.999  |

Figure 4: The lethal curves of Pseudomonas aeruginosa bacteria with induced Rose Bengal at concentrations of 0.2 mg/ml – 0.6 mg/ml at temperature 25 °C in the presence of light. The symbols denote to the experimental values and the lines represent the fitting function.
| Concentration (mg/ml) | Biomass (mg/ml) | Activity (mg/ml) | Growth Rate | Correlation |
|-----------------------|----------------|-----------------|-------------|-------------|
| 0.3                   | 1.04±0.005     | 0.128±0.004     | 33.3±0.33   | 0.999       |
| 0.4                   | 0.99±0.028     | 0.092±0.013     | 35.0±1.86   | 0.987       |
| 0.5                   | 1.01±0.021     | 0.106±0.013     | 43.3±1.34   | 0.993       |
| 0.6                   | 1.05±0.016     | 0.099±0.001     | 29.6±1.10   | 0.994       |

Results are expressed as means ± SD. The values of the parameters were obtained by fitting the data to Eq. 2. *Correlation Factor.

Table 2 shows the fitting parameters of the exposed sample of the Pseudomonas aeruginosa with different concentrations of the Rose Bengal as a photosensitizing additive. Exposure light source with output intensity reaches the samples with about 60J/cm². The table show independent relation between the concentration and the time of the 50% control. The values of the growth rates shown in Table 2 are nearly about 0.1 in the present of Rose Bengal and light. While the values of the growth rates shown in Table 1 are nearly about 0.5 to 0.7 without any additives. The last column shows excellent correlation between the experimental data and theoretical model.

5. Discussion

5.1. Bacteria Growth Curve

Bacterial growth over time can be plotted as a cell number versus time, this is called Growth Curve. The cell number is plotted as the log of the cell number, since it is an exponential function. Regardless of the generation time in a growing culture, the plot of the log of the cell number versus time gives a characteristic curve. In this research, we plotted and fitted the growth curves for three bacteria; Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus. These curves have typically four distinct phases: Lag phase (First part of growth, the cells prepare for synthesis of DNA and enzymes needed for cell division with no increment in cell number and this process extended to few hours); Escherichia coli takes 3 hours, Pseudomonas aeruginosa takes 10 hours, and Staphylococcus aureus takes 4 hours. However, the cells are probably active in metabolizing while preparation for cell division. Depending on the growth medium, the lag phase maybe short or very long. For example, if a culture is a rich growth medium that supplies most of their own amino acids and vitamins, the lag phase will be very long. The cells must activate the metabolic pathways for amino acid and vitamin synthesis and make enough of these nutrients to begin active growth. Exponential (log) phase is the second part of growth, where the culture reaches its maximum rate of growth for specific conditions. In this part, as shown in Figure 1, Escherichia coli takes about 14 hours, Pseudomonas aeruginosa takes 15 hours, and Staphylococcus aureus takes 19 hours to reach its maximum rate, starting from the beginning of lag phase. Stationary phase (third part of the growth curves) takes about 3 hours for this three type of bacteria. In this part, no net increase in numbers and the growth rate equals to the death rate. The death phase (fourth part of growth or the final part). Eventually there was a decline in cell number in this phase. Similarly, we found that Escherichia coli takes 10 hours, Pseudomonas aeruginosa takes 6 hours, and Staphylococcus aureus takes 10 hours in this phase. In this phase, the cells also quickly lose the ability to divide even if they are placed in fresh medium [23]. In our previous study, pre-incubation of MCF-7 cells with iron oxide nanoparticles followed by a static magnetic field exposure significantly (P<0.05) increased doxorubicin-induced cytotoxicity [27].

5.2. Modeling of Growth Curves

The present part reports typical results on the bacterial growth curves fitted to a Modified logistic function model. A new Modified Model for bacterial growth was developed in this study as shown in Figures 1 - 3. The model is described by equations 2 and 3 which consist of useful parameters that characterized the bacterial behaviors in most interesting and important phases; exponential growth and exponential decay, table 1 and 2 [28]. The Modified Logistic model successfully describes the sigmoidal growth curve of Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus under various conditions.
Comparing exponential phase with lethal phase, we can notice that the rate parameter $\alpha (hr^{-1})$ in case of growth phase is lower than that of lethal phase, while the time $\beta$ required to reduce the maximum numbers of colonies to one half of its maximum value were found to be higher in case of growth phase as compared to lethal phase.

The values of the growth rates shown in Table 2 are nearly about 0.1 in the present of Rose Bengal and light. While the growth rates shown in Table 1 are nearly about 0.5 to 0.7 without any additives. This result is consistent with similar finding by Kim, et. al., for wild-type strain MM294 of E. coli cultivated under various conditions, the growth rate without TiO2 and light (normal culture) was 0.43 and reduced 0.40 with TiO2 and light intensity of 12.5 Wm$^{-2}$ [26]. Similarly, the fitted growth curves of Pseudomonas aeruginosa with induced Rose Bengal in presence of light are shown in Figure 4 with very good agreement as those reported by [29, 30]. From the fitted lines with Gaussian Equation, we notice that the faster bacteria to reach the maximum values the broader bandwidths at the half-maximum values could occur. For example, Escherichia coli takes about 14 hours to reach the maximum value with a bandwidth of 12.9 hours, Pseudomonas aeruginosa takes about 15 hours reach the maximum value with a bandwidth of 11.8 hours, and Staphylococcus aureus takes about 19 hours to reach the maximum value with a bandwidth of 8.0 hours. Bandwidths are independent on bacteria group (gram-positive or gram-negative) but it seems totally dependent on the oxygen requirements. An anaerobic bacterium (Escherichia coli and Staphylococcus aureus) takes broader bandwidths than aerobic bacteria (Pseudomonas aeruginosa). This concludes that the growth and lethal rates of anaerobic are much greater than aerobic. This finding is consistent with what has been found by other researchers were Pseudomonas aeruginosa had better degradation rate when there was increase in aeration rate, the biodegradation rate increase too [31].

5.3. Bacteria (E. coli, Pseud. a. and Staph. a.) with Induced Rose Bengal

In this part, the viable counts (Number of Colonies) of Pseudomonas aeruginosa start decreasing with presence of light with induced drug (Rose Bengal) (See Figure 4). Reduction in the bacteria cells are increasing in absence of light (dark) were noticed and recorded. In normal situation, without additives, the number of colonies increases with time. This is probably because the bacteria have been frequently used for the determination of the photodynamic capabilities of sensizers. Due to the general structural and physiological differences between the cell walls of gram-positive and gram-negative microorganisms, the behavior of photodynamic systems may vary depending on the abilities of the sensizers to penetrate membranes and diffuse through the cell walls.

Illumination of Rose Bengal with light produces singlet oxygen ($^{1}\text{O}_2$) which is known to inactivate bacterial cells efficiently and led to cell death. The membrane of the cell was found to be a likely target for the $^{1}\text{O}_2$ reactions in a separated surface-sensitizer system. In contrast with some other sensitizer, the dye did not have to necessarily be bound to the membrane [32]. In addition, there are some clear confirmation that anionic compound penetrates through outer membrane wall and bound inside. Nitzan and co-workers used the polycationic peptide polymyxin B nonapeptide (PMBN), which increased the permeability of the Gram- negative outer membrane and allowed photosensitizer that are normally excluded from the cell to penetrate to a location where the reactive oxygen species generated upon illumination can cause fatal damage [21]. PMBN does not release lipopolysaccharide (LPS) from the cells and expands to the outer leaflet of the membrane allowing photosensitizer such as deuteroporphyrin to penetrate and permitting PDI of Escherichia coli and Pseudomonas aeruginosa. Nitzan, et. al., also reported that an interaction between PMBN and photosensitizer in solution could occur and speculated that this binding assisted the penetration. They found that DP seemed to work much better in concert with PMBN than many other photosensitizers, including porphyrins, phthalocyanines and merocyanine 540 [33]. In comparison with some other finding, polylsine chain of 20 lysine residues did not allow PDI with DP [34]. On our previous study, the bacteria growth curve was compared with control depending on inhibition percentage and it was found higher than control for all different bacteria strains at all concentrations [35, 36].
There is some evidence that photosensitizer can more easily intercalate into double-stranded DNA and cause damage and also Guanine residues have been shown to be the most easily oxidized [37]. The damage may be able to be repaired by various DNA repairing systems [38].

6. Conclusion
In conclusion, viable counts of bacteria incorporated with or/and without photosensitizer such as Rose Bengal are investigated. Typical curves expressed the behavior of the bacterial growth in presence and absence of light. Convenient and general mathematical modeling is obtained to characterize the growth behaviors for selected bacteria.

The conclusions of this work can be summarized as follows:
- Rose Bengal with concentration of 0.2 to 0.6 mg/ml plus enough dose of light (60 J/cm²) are required to cause bacterial destruction.
- The viable counts decrease in presence of light with induced drug (Rose Bengal).
- These models could be considered as the suitable models for describing the growth of bacteria under certain conditions.
- Interestingly, there is a variation in growth phases in all types of bacteria as expected, while no significant change in decay phases.
- Intercomparison study on bandwidths at the half-maximum values were measured and found to be independent of bacteria group (gram-positive or gram-negative) but it seems completely dependent on the oxygen requirements; an anaerobic bacterium takes broader bandwidths than aerobic bacteria. This concludes that the growth and lethal rates of anaerobic are much greater than aerobic.
- Finally, in this study, very good agreements with acceptable correlation were found between the experimental data and theoretical modeling that express the bacterial behavior in general and bacterial growth and lethal in particular.

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