# Efficacy of Bacterial Photodynamic Inactivation with varying Angle and Time of LED Exposure

*by* Suryani Dyah Astuti

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# Efficacy of Bacterial Photodynamic Inactivation with varying Angle and Time of LED Exposure

Suryani D. Astuti[1,a,b], Winda P. Lestari[2,a], Deny Arifianto[3,b] Anggraini Pertiwi[4,b],
[a]Department of Physics Faculty of Sciences and Technology
Airlangga University, Surabaya, Indonesia
[1]suryanidyah@fst.unair.ac.id,[2]wynda_lestari48@yahoo.com
[3]Denyarifianto23@gmail.com, [4]anggrainiprtw@gmail.com

Irawan P. Buditomo[5,b], Nike D.G. Drantantiyas[6,a], Ernie M. Setiawatie[7,c]
[b]Post graduate Program in Biomedical Engineering
[c]Faculty of Dentistry
Airlangga University, Surabaya, Indonesia
[5]aksoro_jowo@yahoo.co.id, [6]grevika@gmail.com,
[7]ernie-m-s@fkg.unair.ac.id

***Abstract*—*Streptococcus mutans* (*S. mutans*) is a bacterium that plays a major role in the occurrence of dental caries. Dental caries is a disease that destroys tooth structure, consequently the tooth becomes perforated as well as the sustainable state of accumulation of *S. mutans* excess in the oral cavity can cause damage to the tooth nerve. One alternative treatment of caries is Photodynamic Inactivation (PDI). PDI is a method of microbial inactivation using light and photosensitizer (PS). The absorption of light energy by PS molecules can produce reactive oxygen species that will damage the bacterial cells. Clinical antimicrobial photodynamic therapy using a light source is generally performed at various angles of irradiation depending on the position of the tooth. The amis of this study is to analyze the effect of LED lamination angle (450.00 ± 0.21) nm for inactivation of S. mutans by adding PS chlorophylls of *Moringa oliefera* leaf with concentration 8 mg / ml. Total Plate Count (TPC) was used to determine the decreasement of bacterial viability in CFU / ml. The ANOVA test showed that the irradiation with variation of 90° exposure angle and 180s exposure time potentially inactivated was significantly different with other treatment variation ($p < \alpha = 0{,}05$). The 90° LED illumination angle with 180 s LED time exposure to produce percentage decrease of bacterial colony (30.30 ± 4.23)% without PS chlorophyll and (46.74 ± 1.67)% with PS chlorophyll. So the angle and duration of exposure time of LED influence PDI efficacy.

***Keywords*—*angle exposure, PDI, LED, chlorophyll***

## I. INTRODUCTION

Dental caries is a disease that destroys tooth structure consequently the tooth becomes hollow. This caused by the excessive accumulation of normal bacteria *Streptococcus mutans* (*S. mutans*) that interact with saliva, as well as the parts that come from food in the oral cavity. If this condition does not get immediate treatment will damage the tissue deeper until the pulp place attached to the tooth nerve [1].

Systemic treatment with antibiotics causes resistance of *S. mutans* to certain types of antibiotics for a long time [2]. So it is necessary to look for alternative methods, one of which is with Photodynamic Inactivation (PDI)[3]. PDI is an in vitro approach to inactivation of microorganisms [4]. The combination of light and certain photosensitizer in PDI will cause photoinactivation in bacteria [5].

The use of photodynamic methods both in clinical and in vitro has been widely investigated. The results of the study [6] [7] [8] show that PDI can decrease the number of bacteria and biofilms resistant to antibiotics. Antimicrobial effects of toluidine blue O (TBO) and a low-energy light emitting diode (LED) after conventional disinfection of NaOC 6% have the potential to be used as additional antimicrobial procedures in conventional endodontic therapy [9].

One of the most widely used light sources in photoinactivation that resides in the photosensitivity absorption spectrum is the Light Emitting Diode (LED). The color of light emitted by the LED depends on the material and condition of the inserted semiconductor, both infrared, visible light, and ultraviolet [10]. The advantages of LEDs, among others, produce heat in small quantities, so it does not cause any photothermal effects. LEDs have been widely used in photodynamic therapy [11].

Photosensitizer (PS) is a light-sensitive substance that plays a role in the absorption of light in PDI. The endogenous PS is produced naturally by bacteria. The absorption of light can be increased by giving exogenous PS. Organic photosensitizers are generally extracted from natural ingredients such as chlorophyll from green plants and photosynthetic bacteria. Chlorophyll has porphyrin-like photophysics properties, with an absorption areas as well as greater intensity of porphyrins [12]. Various PDI studies using LED sources, diode laser and PS chlorophyll have reported successful photoinactivation in microbes [13-15].

The use of LED light sources in clinical antimicrobial photodynamic therapy is generally performed at various angles of irradiation depending on the position of the tooth. This study aims for the effectiveness of antimicrobial photodynamic inactivation at various angles of LED irradiation and exogenous PS chlorophyll Moringa leaf (*Moringa oliefera*).

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## II. METHODS

### Bacterial Strain and Culture Conditions

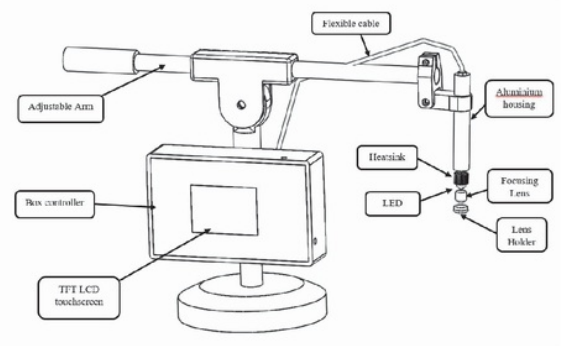
The sample of bacteria strains collected by Faculty of Dentistry, Airlangga University from the tooth of patients was diagnosed with dental caries. The bacteria strains were grown in sterile media of TSB and TSA at 37°C for 24 hours.

### Materials

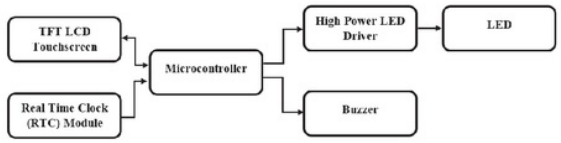
*Moringa oleifera* chlorophyll with a concentration of 8 mg/ml was diluted with the sterile physiological solution. The absorption spectrum of chlorophyll absorption was tested using the Shimadzu UV-VIS 1800 Spectrometer.

### Apparatus chamber for illumination treatment

The PDI LED instrument consists of a controller and LED module. Fig. 1 shows the schematic and work diagram of the apparatus chamber for illumination.




(A)




(B)

**Fig. 1.** (A) Schematic apparatus chamber for illumination treatment of samples; (B) Workflow diagram of apparatus chambers for illumination.

The control box comprises the Atmega328 microcontroller, 3.2" touchscreen LCD for setting the intensity value and the length of time of the LED and LED Driver to adjust the current and the voltage to be streamed to the LED. The LED module consists of a Holder lens as a buffer, focusing lens and LED light source with 750mhA current specifications, 400-500 nm wavelengths output, 0.6mW power output, 3.3V voltage) In the LED module there is also a heatsink to spread the heat from the LED.

### Antimicrobial Effect of Treatments Against *S. mutans* with Total Plate Count (TPC)

To determine the antimicrobial effect of treatments on *S. mutans*, samples were distributed to 4 groups as follow: (1) Groups A(treated with chlorophylls and LED), (2) Group B(treated only with LED), (3) Group C-(negative control, no exposure to either chlorophylls). For each group, the experiment was repeated at least 3 times. All treated with the various time exposure of LED (30 s, 60 s, 90 s,120 s, 150 s, 180 s) s and angle exposure (15˚, 30˚, 45˚, 60˚, 75˚, 90˚). The suspension was planted on Trypticase Soy Agar (TSA) (Merck, Darmstadt, Germany) sterile media and incubated at a temperature of 37°C for 24 hours. After incubation, the number of colony-forming units per milliliter (CFU/ml) was determined. The results were log-transformed and analyzed by analysis of variance (ANOVA) and the Tukey test. A P value ≤0.05 was considered to indicate a statistically significant difference. The percentage decrease in the number of bacterial colonies growth defined as: | (Σ sample colony - Σcontrol colony)/ Σ control colony | x 100%.

## III. RESULTS AND DISCUSSION

### Apparatus chamber for illumination

The characterization of the LED beam power stability aims to determine the stability of the LED power for a certain time. Measurements were made with 4 repetitions over the time range 0-1000 seconds. Fig. 2 shows the graph of the stability of the LED power over time. The result of data fitting shows exponential function $y = y_0 + Ae^{-x/t}$ with $R^2 = 0{,}96$ dan 0,04. Based on the measurement result, the stability of power starts to occur at the time range 300 ± 0,005 s with the fitting results showing that $\frac{dy}{dx} = 0$.

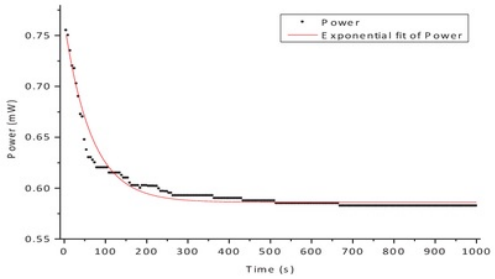



Fig. 2. Graph of the stability LED power versus time exposure

Measurement of the LED power to the distance to determine the output power when the distance is adjusted according to circumstances when irradiation. Fig. 3 shows the graph of the LED power to the LED exposure distance. The fitting results use the exponential function approach $y = y_0 + Ae^{-x/t}$ with $R^2 = 0{,}99$. Based on the graph of the LED power to the distance of exposure indicates that the further the exposure distance, the LED power decreases exponentially. The distance used in the study is 2.5 cm with a

large power (0,266 ± 0,001) mW.At this distance, LED exposure to *S. mutans* cultures with and without PS of Moringa leaf chlorophyll with angle variation and duration of exposure time.

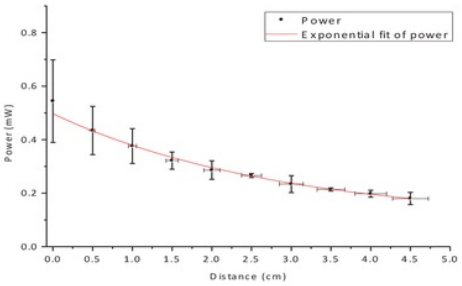



Fig. 3. Graph of LED Power to Distance

**Characterization of *Moringa oliefera* chlorophyll**

PS used in this study is chlorophyll Moringa leaf (*Moringa oliefera*) with a concentration of 8 mg / ml. The absorption spectrum of chlorophyll can be determined by measurement using UV-Visible spectroscopy. The results of the chlorophyll *Moringa oliefera* absorption spectrum test are shown in Fig. 3.

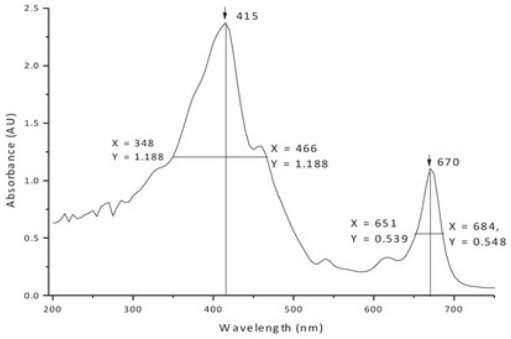



Fig. 4. The graph of *Moringa oliefera* absorbance

The result of plot fitting of chlorophyll approaches Gaussian function with two peaks of the highest absorption spectrum of chlorophyll, ie2,373 ± 0.006 at wavelength 415,0 ± 0,3 nm and 1,106 ± 0,004 at wavelength 670,0 ± 0,3 nm. The results of the chlorophyll absorbance test are in accordance with the research that has been conducted. Bukar et al. 2010[16], which states that Moringa leaf extract has a broad spectrum in blue and red areas. The results of Papageorgious et al., (2000) [17] also show that the spectrum of porphyryn photosensitivity types lies in the wavelength range of 400 nm to 650 nm.

In Table 2 is the Minimum Inhibitory Concentration (MIC) result to estimate microbial populations in suspended *S. mutans* cultures. Fig. 5 is a McFarland graphical conversion of optical density (OD) values against CFU / ml. The fitting results show regression with $R^2 = 0,95$.

Table 2. The Minimum Inhibition Concentration of *S.mutans*

| Dilution to- | The number of colony replication | | | | mean | CFU/ml | Log CFU/ml | Error (±) |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | | | | |
| 1 | 585 | 539 | 545 | 560 | 557,25 | $1,1 \times 10^5$ | 5,05 | 0,18 |
| 2 | 362 | 357 | 351 | 383 | 363,25 | $7,2 \times 10^5$ | 5,86 | 0,16 |
| 3 | 331 | 310 | 315 | 323 | 319,75 | $6,4 \times 10^6$ | 6,81 | 0,18 |
| 4 | 217 | 209 | 198 | 215 | 209,75 | $4,2 \times 10^7$ | 7,62 | 0,11 |
| 5 | 166 | 168 | 141 | 169 | 161,00 | $3,2 \times 10^8$ | 8,51 | 0,01 |
| 6 | 101 | 112 | 110 | 91 | 103,50 | $2,0 \times 10^9$ | 9,32 | 0,39 |
| 7 | 45 | 46 | 53 | 56 | 50,00 | $1,0 \times 10^{10}$ | 10,00 | 0,48 |
| 8 | 39 | 42 | 32 | 49 | 40,50 | $8,1 \times 10^{10}$ | 10,91 | 0,77 |
| 9 | 42 | 45 | 42 | 31 | 40,00 | $8,0 \times 10^{11}$ | 11,90 | 0,70 |
| 10 | 37 | 31 | 35 | 36 | 34,75 | $6,9 \times 10^{12}$ | 12,84 | 0,39 |

*the grey line shows the dilution standard be used in this study

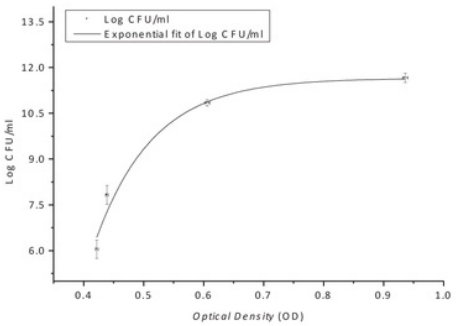



Fig. 5. Graph of McFarland diagram

Fig. 6 shows the graph of decreasing bacterial viability of *S.mutans* due to LED exposure at various times and angles of LED exposure.

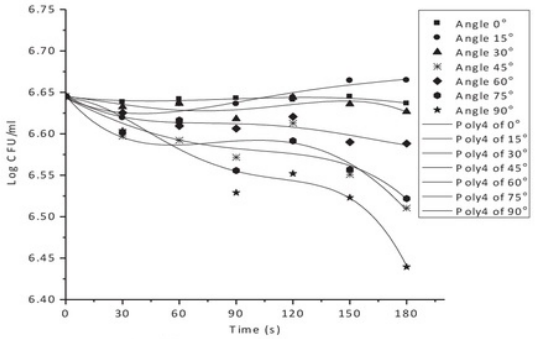



Fig. 7. Graph of *S.mutans* viability at various time and exposure angle of LED

Fig. 8 shows a graph of the decreased viability of *S.mutans* bacteria due to LED exposure at various time and exposure angles of LED with exogenous PS chlorophyll *Moringa oliefera* 8 mg / ml.

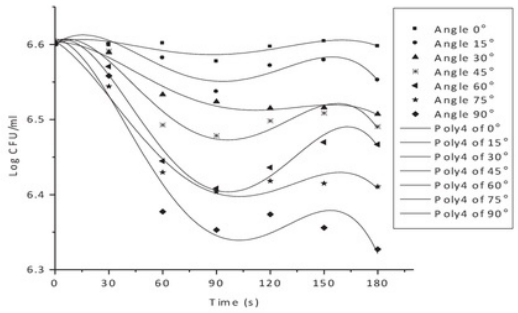



Fig. 8. Graph of *S mutans* viability with exogenous PS chlorophyll *Moringa oliefera* at various time and exposure angle of LED

Based on statistic test result show that irradiation with the variation of radiation angle 90˚ with duration of exposure 180 s significantly different with other treatment ($p < \alpha = 0{,}05$). The 90o LED illumination angle with a duration of 180 seconds produces a percentage of bacterial deaths (30.30 ± 4.23)% without PS chlorophyll, and (46.74 ± 1.67)% with PS chlorophyll.

The reaction at the molecular level due to light irradiance is preceded by a very rapid photophysical event, followed by a primary photochemical reaction, followed by a slow process at the physiological level. The interaction of light through a medium involves the process of refraction, reflection, absorption and scattering[17]. The process of refraction and reflection of light follows the laws of Snellius and the Fresnel law. The above events can be minimized with light applications perpendicular to the target. So that perpendicular light exposure produces the greatest light intensity. The energy of the light photon depends on its wavelength. While the intensity is the amount of power LED emitted per second broad unity. The LED exposure energy meeting is the intensity multiplied by the exposure time. Thus the time of exposure of the LED affects the energy density for bacterial inactivation.

The incident of light energy absorption by PS is the initial stage of photosensitization. The photosensitization process is described in detail by Wilkinson et al (1993) and Plaetzer (2008) [17] [18]. The right wavelength of light radiation will be absorbed by the PS and excite the PS from the ground state (S0) to the singlet (S1) excited state in the nanosecond range and too short to allow significant interaction with the surrounding molecule. In state S1 the PS molecule is unstable, so it will transition one of them via intersystem crossing (ISC) to triplet excitation (T1). The time range in T1 in the micro - milli - second range is long enough for various quenching processes for both energy transfer and electron transfer. Transfer electrons or protons to oxygen or other adjacent molecules to form radical anion or cation [19]. This radical will react with oxygen molecules to generate ROS. The transfer of energy to the oxygen molecule produces a highly reactive dioxygen singlet. Both types of these reactions occur in parallel, and the ratios depend on several parameters, including photosensitizers used and oxygen concentrations [20] [21].

Singlet dioxygen is capable of causing permanent damage to various cell constituents including plasma, mitochondria, lysosomes, nuclear membrane and protein modification. Antimicrobial PDIs with various PS molecules are more effective at disabling Gram-positive compared to Gram-negative bacteria due to differences in cell wall structure [22] [23].

Photodynamic inactivation in dental clinical therapy is very promising. PDI is one of the alternative treatment for multi-drug r esistant bacteria. The use of photodinamic therapy methods on dental offers the effectiveness and selectivity of therapy, meaning it is more effective as an antimicrobial. PDI is a non-thermal photochemical reactions involving the simultaneous presence of visible light, oxygen and color or PS. Some PS have been tested for its ability to efficiently produce ROS that inactivate microbial cell [24].

## IV. Conclusion

The results showed that the irradiation with the variation of 90˚ exposure angle and 180s exposure time potentially inactivated was significantly different with other treatment variation ($p < \alpha = 0{,}05$). The 90° LED illumination angle with 180 s LED time exposure to produce percentage decrease of bacterial colony (30.30 ± 4.23)% without PS chlorophyll and (46.74 ± 1.67)% with PS chlorophyll. So the angle and duration of exposure time of LED influence PDI efficacy.

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