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ANALYSIS ON THE EFFICACY OF PHOTODYNAMIC ANTIMICROBIAL CHEMOTHERAPY IN AQUACULTURE SYSTEMS

Jui-Teng Lin
New Vision Inc. Taipei, Taiwan

Abstract- This paper presents analytic equations for the photoinitiator concentration and the dynamic absorption of the photosensitizer, methylene blue (MB), in an aquaculture system. The modelling is consistent with the measured data showing the dynamic absorption is a decreasing function of the light illumination time due to the depletion of MB concentration. The efficacy and the production rate of reactive oxygen species are proportional to the MB concentration and the initial light intensity.

Keywords - Antimicrobial photodynamic therapy, Photoinitiator concentration, reactive oxygen species, light intensity.

I. INTRODUCTION

Photodynamic therapy (PDT) has been used for various medical applications [1-3] such as polymerization for biomaterials, low-level laser-therapy, light curing in teeth implant and corneal cross linking. Antimicrobial photodynamic therapy (aPDT) has been used for periodontal treatment [4] and chemotherapy in aquaculture [5-9]. However, most of the prior works are focusing on the experimental or clinical studies, much less efforts are performed for the analysis or theory related to the photodynamic processes. We have recently presented a serious of theoretical modellings on the photo-polymerization and cross linking processes [10-13].

In this paper, we will present the comprehensive analysis for aPDT in aquaculture systems which are recently studied experimentally at the Fu-Jen Catholic University, Taiwan, for the bacteria killing and inactivating of white spot syndrome virus [14-16]. The detailed experimental results will be presented elsewhere. The critical issues to be explored in this article include: the dynamic absorption of the photoinitiator, the efficacy of aPDT and the production rate of reactive oxygen species (ROS), and the roles of the light intensity and the initiator concentration.

II. THE MODELING SYSTEM

A light source (either LED or laser) at a wavelength of 660 nm is applied to the absorbing medium of sea water mixed with methylene blue (MB) and bacteria. It was known that the photoinitiator MB will be gradually deleted after the light illumination.

By solving a coupled dynamic equations, The concentration of the photoinitiator $C(z,t)$ is given by an approximate expression [10, 11]

$$C(z,t) \cong C_0 \exp[-XF(z)] \qquad (1.a)$$

$$F(z) = \exp[-2.3\varepsilon_2 C_0 z] \qquad (1.b)$$

Where $X = aI_0 t$, $a = 83.6\lambda\varphi\varepsilon_1$, with φ being the quantum yield and λ being the laser wavelength; and ε_1 and ε_2 are the molar extinction coefficient of the initiator and the photolysis product, respectively. C_0 is the initial initiator concentration; I_0 is the light intensity at the entrance plane (z = 0) of the absorbing medium;. $a = 83.6\lambda\varphi\varepsilon_1$, with φ being the quantum yield and λ being the laser wavelength; and ε_1 and ε_2 are the molar extinction coefficient of the initiator and the photolysis product, respectively.

Analytic approximation for the light intensity is also available [10,11],

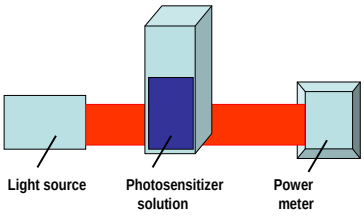
$$I(z,t) = I_0 \exp\left[-A(t)z\right], \qquad (2.a)$$

$$A(t) = 2.3(\varepsilon_1 C_0 + \varepsilon_3) - bC_0 X / b' \qquad (2.b)$$

where $b = 2.3(\varepsilon_1 - \varepsilon_2)$, $b' = 2.3(\varepsilon_2 C_0 + \varepsilon_3)$, $X = aI_0 t$.

III. THE DYNAMIC ABSORPTION

Eq. (2) shows that the dynamic extinction coefficient A(t) is a nonlinearly decreasing function of time (t) at a given z. It also predicts that the photoinitiator becomes more transparent after the UV light illumination, consistent with our observed color change to be shown later. This dynamic feature will be explored by an experimental setup.



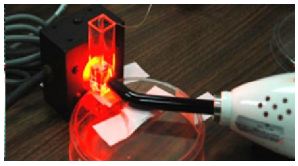


Fig. 1 Experimental setup shows an incident red light and the transmitted light intensity (I) measured by a power meter,

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As shown by Fig. 1, the collimated red light is propagating through a cubic container having a width of 1.0 cm. For a given incident light intensity (I_0), the transmitted light intensity (I) measured by a power meter, is related to the medium dynamic extinction coefficient A(t) by

$$A(t) = \frac{1}{z}\ln\left(\frac{I_0}{I(z,t)}\right) \tag{3}$$

For various initiator molar concentration of 0.05, 0.1 and 0.15 mM, and an incident light intensity of 300 mW/cm^2, the measured the transmitted light powers are used to calculated the dynamic extinction coefficient A(t) based on Eq. (3) for z=1.0 cm with results shown in Fig. 2.

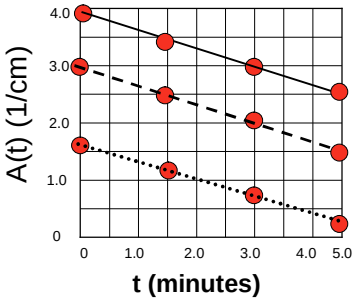

Fig. 2 Measured dynamic extinction coefficient A(t) versus time (t) for various initiator molar concentration of 0.15, 0.1 and 0.05 mM given by solid, dashed and dotted curves, respectively.

The above measured data is consistent with our analytic formula given by Eq. (3) which shows that A(t) is a linearly decreasing function of time (t), when X<<1, and it is proportional to the initiator concentration.

Alternatively, we also measured the dynamic absorption spectra of the MB solution (with 0.1 mM) at various red-light (at 660 nm) illumination time. Fig. 3 shows a linearly decreasing function of time (t) for t<5 minutes and gradually level off for large t. This nonlinear feature is also consistent with Eq. (3). The decreasing of the absorption spectra may be also analysed by Eq. (1) which shows the depletion of MB concentration as a function of time given by $C(z,t) \cong C_0[1-aI_0tF(z)]$, for small X.

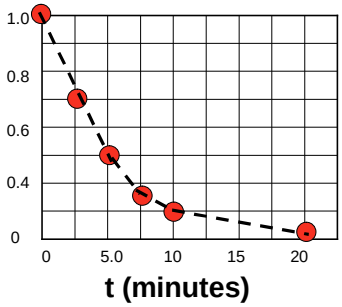

Fig. 3 Normalized dynamic absorption spectra of methylene blue solution after a red-light illumination.

IV. THE OPTIMAL REACTION RATE

Survivable rate of the bacteria in the MB solution is characterized by three factors: the initial light intensity, the MB initial concentration and the light illumination time. The efficacy of aPDT also depends upon the above 3 factors. The local photoinitiation rate of production of the reactive oxygen species (ROS) which kills the bacteria, $R(z,t)$, is represented by [10]

$$R(z,t) = 167.2\lambda\varepsilon_1\phi I(z,t)C(z,t) \tag{4}$$

which is given by, from Eq. (1) and (2),

$$R(z,t) \propto I_0C_0\exp\left[-2.3(\varepsilon_1C_0+\varepsilon_3)z\right]\exp[-X(F-bzC_0/b')] \tag{5}$$

Above equation indicates that the photoinitiation rate is proportional to the product of $\varepsilon_1C(z,t)$ and the light intensity which is a competing deceasing function of $\varepsilon_1C(z,t)$. Therefore one may expect an optimal value of ε_1C_0 (at a given z) for a maximal photoinitiation rate caused by the balance of the two competing factors. Furthermore, R is a decreasing function of time and reduces to zero when the initiator concentration of completely depleted, or when C(z,t)=0. The time dependent of R is given by

$$R(z,t) \propto I_0C_0\exp[-X(F-bzC_0/b')] \tag{6}$$

which shows an exponentially decreasing of time.

V. SURVIVABLE RATE OF BACTERIA

The bacterial residual percentage by aPDT is measured and shown in Fig. 4 for various MB initial concentration of 0.01, 0.05 and 0.1 mM and illuminated by a red light intensity of 700 mW/cm^2 [14].

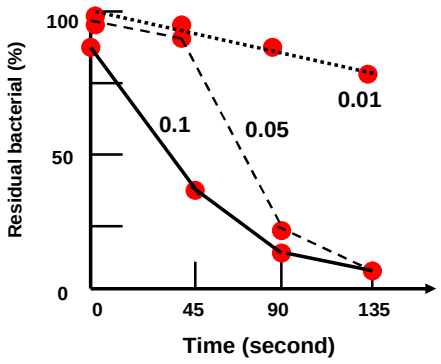

Fig. 4 Measured bacterial residual percentage for various MB initial concentration of 0.01, 0.05 and 0.1 mM with a red light at 660 nm and intensity of 700 mW/cm^2 [14]

The above bacterial residual percentage may be easily analyzed as follow. By Eq. (6), the reaction rate ROS is proportional to the MB concentration, therefore lower bacterial survivability is expected in high concentration. In addition, smaller slope of the bacterial residual curve is

expected at longer illumination time, as shown by the exponential decreasing of Eq. (6).

VI. CONCLUSIONS

In this paper, analytic equation for the dynamic absorption is presented and compared with the measured data showing a decreasing function of time. We also show the depletion of MB concentration after the light illumination. The efficacy of aPDT and the production rate of reactive oxygen species (ROS), which kills the bacteria, $R(z,t)$ is proportional to the MB concentration and the initial light intensity.

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