The Experimental Research and the Quantum and Thermotics Theory Analysis of Lethal Effect of CO$_2$ Laser Irradiation on Yeast Suspended in Liquid

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Abstract. In this paper, a lethal effect experiment was carried out, in which the biological cells of suspension-industrial saccharomyces cervisiac Sachromyce 2.1189 in liquid was irradiated by CO$_2$ laser. The experiment results were analyzed by means of quanta theory and thermotics theory. The theoretic analysis results can explain well the experimental results of the lethal effect.

1. Introduction

Laser breeding has been widely used. But in breeding experiments, it is difficult to control the radiation dose. Shorter laser irradiation time and lower irradiation laser power can’t lead to variations and reach breeding purposes. However, overdose cause their genetic destructive denaturation, or simply to make living biological cells dead (lethal effects) and lead to breeding failure. In breeding experiment of CO$_2$ laser irradiation on yeast, we found bacteria had more deaths at the irradiation time of 26s, but closing to the irradiation time of 26s there is a high mutation rate[1]. Therefore, the lethal effect of laser irradiation research is extremely important in breeding and laser medical applications. In laser breeding microbial genetic mutation, the common method is to make a suspension with microbial cells, and then use the laser irradiation, in which CO$_2$ laser is used. As the wavelength of CO$_2$ laser is in infrared wave band, the major laser effect should be thermal effect. Repeated experiments showed that CO$_2$ laser irradiation could result in higher temperature in suspension, and longer radiation time, there is cell death. Through evaluating and testing some dates of lethal effect, we have a further understanding of the lethal effect of laser irradiation on yeast and its mechanism. We make microscopic mechanism analysis of experimental results of the lethal effect with quantum and thermotics theory.

2. Experiment

Experimental material is industrial alcohol from sugar cane molasses sacchromyces cerevisiae 2.1189, and culture media are YPD and $12B$, diluted sugar cane molasses. The $V=0.2ml$ yeast suspension is in sterile conditions of amperes bottle irradiation. The CO$_2$ laser power is 10W, the expanded beam spot diameter is 0.8cm. We set up six parallel time periods , which are 5s, 10s, 15s, 20s, 25s, 30s.
irradiation time of comparison group is (CK) 0s. The bacterial suspension, which is irradiated by CO₂ laser, is cultivated by spread utensil diluting with normal saline. The comparison groups are set up. Bacterial numbers are counted after 3 days cultivating at 28 °C, the results are shown in Table 1. The results of experiments will be analysis theoretically. We can obtain the relation of lethal rate of the cells and the irradiation time of CO₂ shown in the figure 1.

Table 1. The statistical results of lethal effect of CO₂ laser irradiation on the Sacchromyces cerevisiac (The average results of three experiments).

| Group | Irradiation time/s | Tem/°C | Number of yeast cells/ml | Lethal rate/% |
|-------|-------------------|--------|--------------------------|---------------|
| I     | 5                 | 48     | 8.86 × 10⁷               | 32.37         |
| II    | 10                | 54     | 5.74 × 10⁷               | 56.18         |
| III   | 15                | 58     | 3.72 × 10⁷               | 71.60         |
| IV    | 20                | 61.5   | 1.66 × 10⁷               | 87.32         |
| V     | 25                | 63     | 3.76 × 10⁴               | 99.79         |
| VI    | 30                | 63.5   | 0.00                     | 100.0         |
| CK    |                   |        | 1.31 × 10⁸               | 0.00          |

Figure 1. The relation of lethal rate and irradiation time by CO₂ Laser.

3. The macroscopic thermology theory of the experimental results
CO₂ laser irradiates the suspension from top to bottom, which energy is absorbed in the suspension. The relationship between laser energy flux density $I$ and the depth of the laser entering into the suspension is given through Lambert’s law [2] (simple terms, omitted nonlinear effect)

$$I = I_0 e^{-\gamma y}$$  \hspace{1cm} (1)

where $I_0$ is the energy density of the laser at the suspension surface, and $\gamma$ is the coefficient of cell body's absorbing CO₂ laser. If the reflector bottom is taken into account, then (1) should be

$$I = I_0 (e^{-\gamma y} + \eta e^{\gamma H})$$  \hspace{1cm} (2)

where $\eta = \beta e^{-2\gamma H}$, $\beta$ is the reflection coefficient of the bottom and $H$ is the depth from cell layer to the bottle bottom. Taking one layer of cells of $y-y+dy$, then the laser energy of the layer by cells absorbing in the unit time is

$$dQ_y = -sdl = s I_0 (e^{-\gamma y} + \eta e^{\gamma H})$$  \hspace{1cm} (3)
where \( s \) is the cross-sectional square of the liquid column. As the liquid absorb the laser energy, the temperature distribution of the liquid changes. Thus the heat flow appeared in liquid from top to bottom, and due to heat flow the net heat enhancement in the liquid layer \( y-y+dy \) is

\[
dQ = -s \frac{dq}{dy} dy
\]  

(4)

where \( q \) is the heat flux intensity. By the Fourier theorem (Strictly speaking, the application of Chester revising theory of heat conduction, Fourier heat conduction experiment is only an approximation [3]) it has

\[
q = -\kappa \frac{\partial T}{\partial y}
\]  

(5)

In equation (5), \( \kappa \) is the coefficient of the liquid thermal conductivity, and \( T \) is the temperature of the liquid. Therefore

\[
dQ = \kappa s \frac{\partial^2 T}{\partial y^2} dy
\]  

(6)

Considering (3), (6), the increased energy per unit time in the liquid layer \( y-y+dy \) should be

\[
dQ = dQ_1 + dQ_2 = s[I_0 \gamma (e^{-\gamma y} + \eta e^{\eta y})] + \kappa s \frac{\partial^2 T}{\partial y^2} dy
\]  

(7)

On the other hand, the relationship between the rate of temperature change to the time in the liquid \( y-y+dy \) layer and \( dQ \) is

\[
dQ = c \rho s \frac{\partial T}{\partial t} dy
\]  

(8)

where \( c \) and \( \rho \) are respectively, the specific heat and density of liquid. Considering (7), (8),

\[
\frac{\partial T}{\partial t} - \alpha^2 \frac{\partial^2 T}{\partial y^2} = b(e^{-\gamma y} - \eta e^{\eta y})
\]  

(9)

where \( \alpha^2 = \kappa / cp \), \( b = I_0 / c \rho \). Equation (9) is the heat conduction equation that CO\(_2\) laser irradiated to the suspension effect. The role of the right side of the equation (9) shown is equivalent to the heat source that irradiating action of suspension. Given that the temperature \( T_0 \) of the suspension in front of laser irradiation is uniform, the following initial conditions is

\[
T(0, y) = T_0
\]  

(10)

Generally speaking, the irradiation time of CO\(_2\) laser acting to the suspension is short. The impact of suspension of the border can be neglected. Thus, according to (9) and (10) two equations relation that the temperature distribution of the suspension changes with time.

Neglecting the case of the boundary effect, the equation (9) in the following initial conditions of (10) has the following solution [4].

\[
T(y, t) = \int_0^t \int_{-\frac{T_0}{2a\sqrt{\pi}}}^{\frac{T_0}{2a\sqrt{\pi}}} \frac{e^{-\alpha y^2}}{\sqrt{2\pi}} d\zeta \int_0^\frac{b}{2a} \frac{e^{-\alpha t(\tau - \zeta)}(e^{-\gamma \zeta} - \eta e^{\eta \zeta})}{\sqrt{2\pi}} d\zeta d\tau
\]  

(11)

The first integrality of the equation

\[
\int_{-\frac{T_0}{2a\sqrt{\pi}}}^{\frac{T_0}{2a\sqrt{\pi}}} e^{-\alpha y^2} d\zeta \to T_0 \int_{-\frac{T_0}{2a\sqrt{2\pi}}}^{\frac{T_0}{2a\sqrt{2\pi}}} e^{-\alpha y^2} d\zeta = T_0
\]  

(12)

The second integrality of the equation
Thus it can be solved that

\[ T(y,t) = T_0 + \frac{b}{a' \gamma^c} (e^{-\eta} - \eta e^{\eta}) (e^{a' y_{c} t} - 1) \]  

In fact, the absorption of suspension by the CO₂ laser is very strong, while the reflection of the glass bottom for the CO₂ laser is not too strong. Thus \( \eta \) is small, and therefore \( \eta e^{\eta} \) can be neglected in comparison with the experiment, namely

\[ T(y,t) = T_0 + \frac{b}{a' \gamma^c} e^{-\eta} (e^{a' y_{c} t} - 1) \]  

Considering (15), it shows that the temperature becomes higher along with the increasing of the laser irradiation time, and the temperature becomes lower and lower along with the increasing of the liquid depth.

In the suspended state, the yeast cells distribute in the liquid is uniform. Supposing \( T_c \) is the lethal temperature. Along the increasing of the laser irradiation time, \( T_c \) is moving from top to bottom. And suppose \( H \) is the total depth of the liquid, \( y_{c} \) is the liquid depth corresponding \( T_c \), then when the irradiation time is \( t \), considering (15), the death rate could be obtained

\[ \frac{y_{c}}{H} = \frac{1}{\gamma H} \ln \frac{b(e^{a' y_{c} t} - 1)}{(T_c - T_0)a' \gamma^c} \]  

Using the following data [4],

\[
\begin{align*}
    s & = 5.0 \times 10^{-3} m^2; \quad \nu = 2.0 \times 10^{-7} m^3; \\
    \rho & = 1.07 \times 10^{3} Kg/m^3; \quad \kappa = 0.403 J / mKs; \\
    c & = 4.22 \times 10^{7} J / Kg; \quad \gamma = 1.058 \times 10^{3} m^{-1}; \quad T_0 = 25^\circ C; \quad b = I_0 \gamma / c \rho = 23.43(ks^{-1}); \\
    a' & = k / c \rho = 8.925 \times 10^{2} (m^2 s^{-1})
\end{align*}
\]

Using the above data and in a set of experimental values in table 1 into (16),

\[ T_c = 64^\circ C = 337 K \]  

In order to further analyze the mechanism of our results, we will address below with the quantum theory.

4. Analysis of experimental results with the quantum theory

From the microcosmic view, the heating effect of biological molecules is, in fact, the chain of key biological molecules thermal vibration effects. In accordance with Fröhlich’s theory [5], the living biology molecular system is a very high coherence and high ordered system, hence it can be regarded as a related form of galvanic oscillator, while the oscillator frequency is in infrared wave band. So it is easy to be prone to resonance absorption for CO₂ laser and result in a strong warming effect to make the biological cells dead as CO₂ laser irradiation. We will make the microscopic mechanism of the lethal effect of CO₂ laser irradiation on yeast for specific analysis as follows. According to what is mentioned analyses, we can take the bimolecular system as a harmonic oscillation system. The partition function of the bimolecular system is [6]
\[ Q = [1 - \exp(-\hbar \omega/kT)]^s \]  (18)

Where \( s \) is the freedom of bimolecular vibration, \( k \) is Boltzmann constant, \( T \) is the temperature of system. The average absorption energy that each bimolecular absorbs laser photon is

\[ E = \langle n \rangle + \hbar \omega \]  (19)

Where \( \langle n \rangle \) is the average amount of absorbed laser photon by each molecule, \( \omega \) is laser frequency (notice: resonance absorption is major). According to statistical physics, the average energy of each harmonic oscillation system is:

\[ E = kT \frac{\partial \ln Q}{\partial T} \]  (20)

From (18) (19) and (20) we can obtain the temperature-rising formula of bimolecular system by laser heating.

\[ \Delta T = \frac{\hbar \omega}{k[\ln(1 + \frac{s}{\langle n \rangle})]^2(\langle n \rangle + s)} \]  (21)

From the above formula we can get that the temperature rising \( \Delta T \) increases easily when the freedom \( s \) is little, but \( \Delta T \) increases when absorption photos number of bimolecular \( \langle n \rangle \) increases. The \( \langle n \rangle \) is related to the laser intensity and laser-bimolecular interaction. Actually \( \langle n \rangle \) is related to the absorption coefficient of bimolecular for the laser photon. Owing to the complexity of the interaction of laser-bimolecular and of bimolecular self, the absorption coefficient of bimolecular is decided by many factors. We will analysis from tow respects.

The research of biophysics demonstrated that the bimolecular (e.g. DNA and protein) has semiconductor property. Hence we discussed the absorption coefficient of bimolecular for laser photon by means of band theory in solid quantum theory. The absorption coefficient \( \eta \):

\[ \eta = \frac{\hbar \omega}{I} \]  (22)

Where \( \langle n \rangle \), is absorption photon number in unit time and unit volume, obviously related to \( \langle n \rangle \); \( I \) is the laser energy density of incident, obviously relate to laser intensity; \( \eta \) is a macroscopic value which can be macro-examined; \( \langle n \rangle \) is microscopic physical quantity. Now we analyze \( \langle n \rangle \), from micro-mechanism. According to band theory [9]

\[ < s > = \frac{2\pi e^2 A_0^2}{m^2} \sigma \]  (23)

Where \( \sigma \) is the quantity related to scattering cross-section. Then we can obtain the absorption coefficient \( \eta \):

\[ \eta = A \sqrt{\frac{(2\mu)^3(h\omega - E_g)}{h \omega}} \]  (24)

Where \( E_g \) of formula (24) is width of forbidden band, \( E_g \) of biomolecule (DNA and protein) compute by means of the methods of HMO, Hiickel, etc(in general \( E_g \sim 10eV \)). According to (24) and the under help of the relevant formulas in references [7], irradiation time of heating unit \( \Delta T / T_0 \) can be obtained through a series of calculations. Temperature ratio has the relationship with the laser power,

\[ \Delta T = [A(\alpha)P + B(\alpha)P^{-1} - \frac{\hbar \omega}{k}] \delta(P - r P_0) \]  (25)

Where the order of magnitude of \( \alpha \) take \( 10^1 \) or \( 10^2 \) when molecule size take \( 1 \times 10^{-10} \text{m} \), \( h \omega \sim 10^{22}, P \sim 10W \) . That is to say, when \( \alpha \) is a determined value, \( A(\alpha) \) and \( B(\alpha) \) are two related constant, while \( h \omega/k \) is small which could be omitted, \( \delta(P - r P_0) \) is the \( \delta \) function,

\[ \delta(P - r P_0) = \begin{cases} 1, & p = r P_0 \\ 0, & p \neq r P_0 \end{cases}, (r > 1) \]  (26)
According to (25), it could be seen that the increase of temperature with the laser power promoted the overall non-linear relationship. When \( P \) is big, by the first part of (25), \( \Delta T \) and \( P \) overall linear relationship, while \( \alpha \)'s value is related to the thermal motion of molecules entropy, while \( \Delta T \) is the increased temperature in unit irradiation time, with time \( t \) increasing, the increased temperature is raised, when the temperature increases, thermal motion intensify, the corresponding entropy increase. \( \alpha \)'s value becomes smaller. It could explain in table 1 when the temperature is higher, the temperature rise ration becomes slower. Thus we use quantum theory to explain the microscopic mechanism of our experimental results well.

5. Conclusions
1. \( \text{CO}_2 \) Laser irradiation on yeast lethal effect of the laser-induced breeding is significant.
2. Our theoretical analysis and experimental results are consistent. Here it should be stated that since the bimolecular interaction of the laser and the complexity, our research is only preliminary exploration. In addition, in heat transfer theory; we still applied the Fourier heat equation, which is slightly higher than the first. One of the authors in the reference [3] pointed out the limitations of the Fourier heat equation, and the process used in solving a number of similar, their approximate level is still to be improved [8], we make a special explanation here. In addition, using the Quantum theory to analyze the thermal effects of biology of laser is only an exploration; the method is worthy of discussion. In short, there are many areas worthy of further study about theoretical analysis of this article.

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