Journal of Optoelectronics and Advanced Material Vol. 4, No. 1, March 2002, p. 129 - 134

COMPETING PHENOMENA IN LASER EFFECTS ON THE GROWTH OF YEAST CELL COLONIES

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The low-energy laser radiation induces various effects on biological systems. While the thermal mechanism of the interaction of the laser radiation with the biological systems is well known, not the same is the case for the non-thermal effects i.e. non-concerning the temperature increase. This paper put in evidence two competing phenomena in laser effects induced by low energy laser radiation on the growth of the yeast cell colonies and presents some considerations regarding the mathematical model of the process.

(Received May 2, 2001; accepted November 15, 2001)

Keywords: Yeast cell colonies, Laser effect

1. Introduction

The concept of "biostimulation" occurring at low energy levels of laser radiation is already generally accepted, despite some controversy concerning especially the mechanisms by which the biological response appears.

The study of interaction between electromagnetic waves and the living systems involves many physical and biological aspects. On the one hand, it was demonstrated that there is a "threshold stimulus", in the sense that the monochromatic signal carrier becomes stimulus only if its energy exceeds a threshold value [1]. On the other hand, all observed biological effects of this signal action originate from changes in cellular membrane potentials, resulting from changes in membrane permeability and microviscosity [2]. These changes are caused by the action of the excitation that provokes charge separations, which generate concentration and potential gradients [3].

Also, the electromagnetically-induced biological effects may be characterised as adaptive or compensatory. The electromagnetic action determines an integrative response: after the electromagnetic wave detection, information is communicated to the control system, which then activates the physiological mechanisms to determine a compensatory response.

Many of these effects show different ways of the manifestation, due to the competition between two antagonistic processes, as found in our experiments.

2. Theoretical background

The dynamics of a population, n, (a cell culture, for example) can be described in a mathematical model [4] by the equation:

$$\frac{dn}{dt} = k(n)\cdot n + F(t) \tag{1}$$

where:

$$k(n) = \alpha - \beta\cdot n \tag{2}$$

with α, β constants and F(t) a general formation that describes of the external factors.

The relation (2) means that the growth rate of the population is a subtraction between the population growth factor and the population decrease factor (inhibitor factor).

On the one hand, the population growth factor can be considered constant (in case of our experience, this factor is the mitotic division factor): $f_c = \alpha$.

On the other hand, the inhibitor factor increases with the cell number, with the condition (achieved in the experience) that the food resources are limited, the culture medium being the same, from the sow and without some supplementary food allowing: $f_s = \beta \cdot n$

If α is negative, the stationary solution is $n_0 = 0$, i.e. the population extinction. For $\alpha > 0$, the population firstly increases exponentially, to reach at a given moment, a saturation value, $n_s = \frac{\alpha}{\beta}$. That second situation characterises the growth process of the cell culture in our experiences. The experimental results confirm the theoretical evolution, given by equation (1), plotted in Fig. 1, curve 1.

Excepting the influence of the external factor, the equation (1) becomes:

$$\frac{dn}{dt} = \alpha \cdot n - \beta \cdot n^2 \tag{3}$$

that is a differential, inhomogeneous, first order Bernoulli equation, generally given as:

$$\frac{dy}{dx} + m(x) \cdot y = n(x) \cdot y^{\delta} \text{ with } \delta \neq 0 \text{ and } \delta \neq 1 \tag{4}$$

By substituting $z(x) = y^{1-\delta}$, the equation reduces to a linear one:

$$y'(x) + p(x)y(x) = q(x) \tag{5}$$

with the general solution:

$$y(x) = Ce^{-\int_{x_0}^{x} p(t)dt} + \int_{x_0}^{x} q(t)e^{-\int_{x_0}^{x} p(s)ds} dt \tag{6}$$

Making the variable changes $y \rightarrow n$ and $x \rightarrow t$, with $p(t) = \beta$, $q(t) = \alpha$ and $\delta = 2$, and the initial condition $n(0) = 0$ (in fact, n_0 cannot be strictly equal to 0, but it is very small, near the zero value), the solution is:

$$n(t) = \frac{\alpha}{\beta} \cdot \frac{1}{1 + Ce^{-\alpha t}} \tag{7}$$

where

$$C = \frac{\alpha}{\beta} \frac{1}{n_0} - 1 \tag{8}$$

The temporal evolution, $n = n(t)$ is plotted in Fig. 1, curve 1.

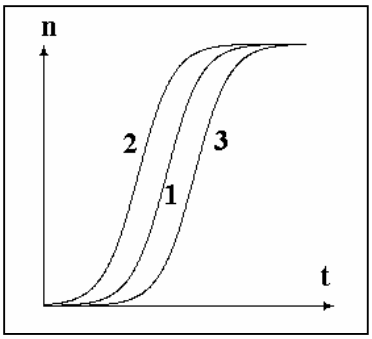

Fig. 1. Theoretical time-evolution of the number of cells in a colony: for a given C value (curve 1) for C' > C (curve 2) and for C″ < C (curve 3) (C' > C) - curve 2, and C" value (C" < C) - curve 3.

If the factor C decreases or increases p times, according to the relation:

$$n(t) = \frac{\alpha}{\beta} \cdot \frac{1}{1 + pCe^{-\alpha t}} \tag{9}$$

then the plotted curve is qualitatively the same, but translated on the t-axis, in the positive sense if $p < 1$ (curve 3, Fig. 1), or in the negative sense if $p > 1$ (curve 2, Fig. 1) (inhibition, respectively acceleration of the growth process).

3. Materials and methods

Among the fungi, a remarkable importance has *Saccharomyces cerevisiae*, for the wine's qualities and therefore we choose this yeast to determine the action of the laser visible light on it.

The material consisted in *Saccharomyces cerevisiae* yeast culture and was prepared as it follows: the young yeast cell culture (18 ÷ 24 hours) in liquid medium (YEPG: yeast extract – 10 g/ℓ, peptone – 10 g/ℓ, glucose – 20 g/ℓ, distilled water tile 1 ℓ) was sowed on Petri dishes with solid medium (yeast extract – 10 g/ℓ, peptone – 10 g/ℓ, glucose – 20 g/ℓ, agar – 25 g/ℓ, distilled water tile 1 ℓ), to obtain isolated colonies (temperature = 28 ÷ 30 °C). The colonies were irradiated with a continuous wave laser beam from a He-Ne laser (λ = 632.8 nm, P = 6 mW), at various exposure times. A laser diode (λ = 670 nm, P = 4 mW) was also used.

From each irradiated colony, a sample was sowed in a liquid culture medium (YEPG). The samples were left at room temperature, 18 ÷ 20 °C, in closed test tubes, for the cell multiplication. To measure the growth rate-time dependence, one can make the following considerations: the cellular growth rate is proportional to the yeast cell density in culture medium at that moment. Let's consider the extinction of the non-sowed medium equal to zero and the extinction of the culture medium in which was sowed a yeast strain, at a time t, E (t). The Beer law of the light attenuation in a solution is $E = \varepsilon cz$, where ε - the extinction coefficient and c – the concentration of the absorbent in solution and z the depth into solution. Obviously, the extinction E being proportional to concentration, c, of the solution, measuring the extinction of the solution samples at different times, one can find out the variation of the concentration, i.e. the variation in time of the number of yeast cell in colony.

The extinction-time dependence for every sample irradiated at different doses (after stirring for solution homogenisation) was determined, in order to point out the possible influence of the irradiation on the yeast culture growth. The determination of the extinction was made with a photocolorimeter SPECOL-Carl Zeiss Jena, at the wavelength for the maximum transparency of the solution (about 700 nm).

The samples were irradiated as follows:

Table 1.

Sample	1	2	3	4	5	Control
Exposure time (min)	1	3	5	8	15	0

The experiment was repeated using a laser diode, all other conditions remaining the same, in order to determine the specificity of the radiation action, knowing that different laser radiation may have different bio-effects.

4. Experimental results

The results of the extinction measurements at different times are presented in Table 2 and plotted in Fig. 2 (the relative extinction of the solution samples at different times; the culture solution extinction is related to the extinction of the non-sowed culture liquid medium) for the irradiated samples with the He-Ne laser radiation, and respectively in Table 3 and Fig. 3 for the irradiated samples with laser diode radiation.

The results were practically the same, consisting in the inhibition of the cell division and colony growth.

Table 2. The relative extinction of the samples irradiated with the He-Ne laser radiation.

Sample		time (h)							
		6	11	17	21	26	30	35	45
Control	EXTINCTION	0.005	0.045	0.17	0.5	0.62	0.63	0.67	0.67
1		0	0.005	0.08	0.26	0.51	0.61	0.63	0.66
2		0.001	0.001	0.1	0.23	0.49	0.61	0.62	0.64
3		0	0.03	0.1	0.26	0.52	0.64	0.63	0.67
4		0.01	0.03	0.15	0.37	0.56	0.62	0.64	0.7
5		0.005	0.01	0.03	0.13	0.345	0.53	0.59	0.61

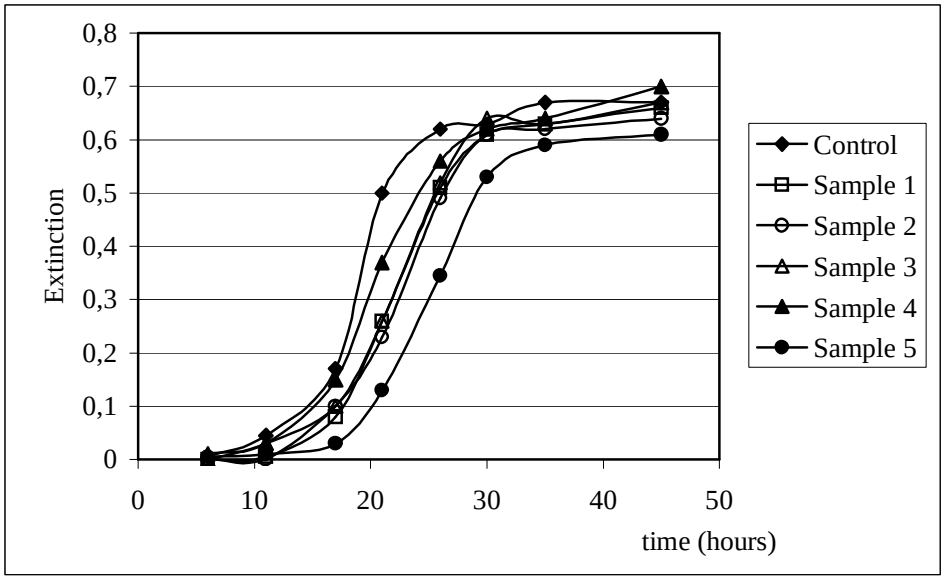

Fig. 2. The time-variation of the relative extinction of the samples irradiated with He-Ne laser radiation.

Table 3. The measured extinction of the samples irradiated with the laser diode radiation.

Sample		time (h)							
		0	10	16	20	25	29	34	44
Control	EXTINCTION	0	0.01	0.03	0.175	0.44	0.63	0.65	0.68
1		0	0.01	0.01	0.1	0.27	0.52	0.64	0.66
2		0	0	0.005	0.07	0.22	0.47	0.6	0.61
3		0	0	0.02	0.16	0.35	0.54	0.6	0.62
4		0	0.01	0.015	0.17	0.31	0.55	0.61	0.64
5		0	0.005	0.005	0.04	0.18	0.43	0.6	0.64

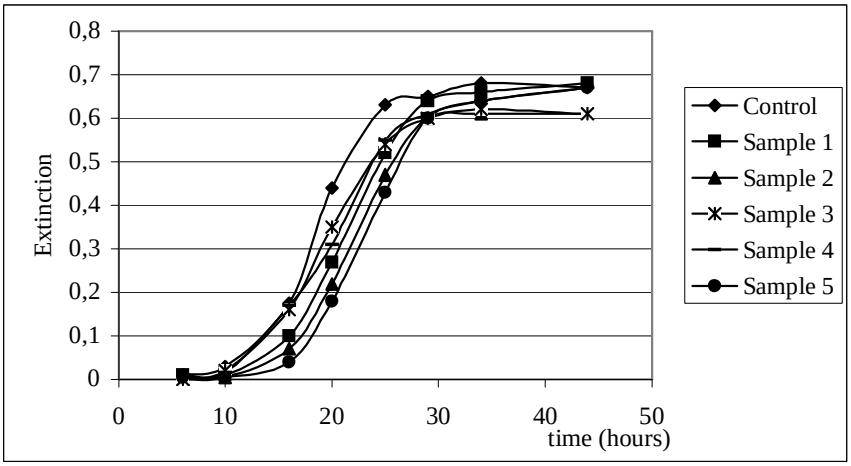

Fig. 3. The time-variation of the relative extinction of the samples irradiated with laser diode radiation.

5. Discussion and conclusions

As we can see, from Fig. 1, in comparison with Fig. 2 and Fig. 3, the laser radiation has an evident inhibition effect on the cell culture. The experimental curves in Fig. 2 and Fig. 3 are curve 1 – type for the control samples (non-irradiated) and curve 3 – type for the irradiated samples. The result is the same for both types of laser radiation.

Some authors [5, 6] also pointed out the inhibitory action of the laser radiation on the colony growth. However, in other experiments [7-9], it was pointed out, on the contrary, a stimulation of the colony growth by the laser radiation action. The response of the biological system to the laser radiation in visible and near IR regions is due to the physical and chemical modifications in the photoacceptor molecules, components of respiratory chains [10, 11]. Such modifications can be: alteration of redox properties and acceleration of electron transfer, changes in biochemical activity due to the transient local heating of the chromophores, one-electron auto-oxidation.

Different reaction channels can be activated to achieve the photobiological macro-effect. A cellular cascade of biochemical reactions follows the primary physical and/or chemical changes induced by light in photoacceptor molecules, which do not need further light activation. These actions are connected with changes in homeostatic cell parameters.

The crucial process is the alteration of the cellular redox state: an increase of the oxidation is associated with the cell vitality stimulation, and a reduction of the oxidation is associated with inhibition. The cells with a lower pH than the normal i.e. a state in which the redox state is reduced, are more sensitive to the stimulating light action than the cells with normal parameters.

The explanation of the dual behaviour of the cell culture may be offered by the fact that, in the visible region, two competing processes determine the stimulation or the inhibition of biological activity. On the one hand, the formation by photosensitization of reactive oxygen species occurs, which stimulate the redox activity of the respiratory chain. On the other hand, there is the intramolecular electronic-vibrational energy transfer from an endogenous photosensitiser to an enzyme of the respiratory chain, thereby bringing this enzyme into an inactive configuration and paralysing the respiratory chain [12]. That circumstance explains the results of our experiments.

In our experiments, the second process is more intense, leading to the increase of C factor (that means a decrease of β factor) and to the inhibition of the cell division. Comparing our experimental conditions with the other ones, (e.g. Ref [7]), we can see that it was used the same type of biological material, the same laser type and the same exposure dose. The differences, that determine also the contrary results, consist only in the irradiation procedure and in the state of the cell cultures. Thus, in our experiments, the irradiation was performed on cells in solid culture medium, while in the other ones, the irradiation was performed on the cells in liquid culture medium.

Another difference is that in our experiments the irradiation was performed in a unique dose at high intensity (direct exposure of the colony in the laser beam) while in the other ones the irradiation was performed continuously, in all growth period (48 hours approximately), but at lower

radiation intensity, due to the dispersion of the light in the liquid medium with a larger volume than the colony volume in the solid medium. According to the Roscoe-Bunsen reciprocity law, this aspect cannot determine a different biological response, resulting that the only possible cause for the different behaviour of the biological system is due to the different culture medium in that are the irradiated cells. We can assume that, in liquid medium, the permeability of the cell membrane is increased, that allows for more intense changes between the environment and the cell, that determine the stimulation of the redox activity of the respiratory chain (a comportment described by the curve 2 in Fig. 1). The irradiation in solid medium determines the exceeding of the threshold that activates the inhibition process of the enzyme that blocs up the respiratory chain (a comportment described by the curve 3 in Fig. 1).

We can also see that one cannot point out an appropriate relation between the exposure dose (exposure time) and the inhibition level of the process. This can be explained by the fact that the inhibitor factor do not act by a mechanism "more intense factor → more intense inhibition", but there is a threshold of the inhibitor factor, in the sense that if the exposure dose in under the value of that threshold the inhibition does not occur, and if it exceeds that threshold the inhibition occurs with a relative independence (in some limits) on the exposure dose.

The existence of this kind of threshold can be sustained by the fact that, if the exposure time exceeds 8 minutes, the irradiated samples do not exhibit properties modifications (alcohol generation power, acidity) [13].

6. Conclusion

The laser radiation shows an inhibition effect on the yeast cell culture. Two competing processes determine the stimulation and the inhibition of the cell activity: the increase of oxidation and the decrease of oxidation capability, respectively. The inhibition of the redox activity in the respiratory chain is the predominant factor in our irradiation experiments.

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